JOURNAL OF DAIRY SCIENCE

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Vol. XXXI, No. 9, September, 1948

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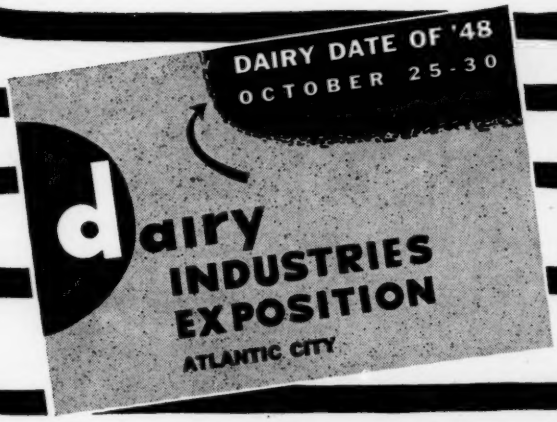

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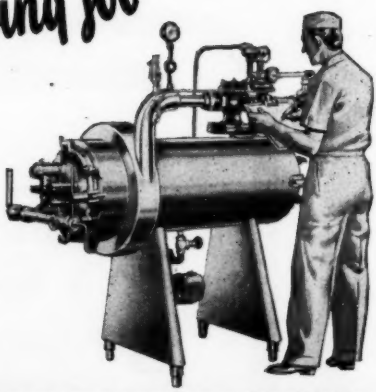

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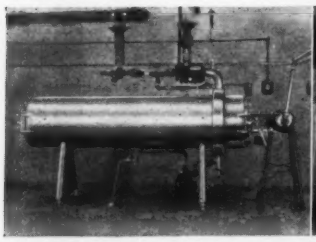

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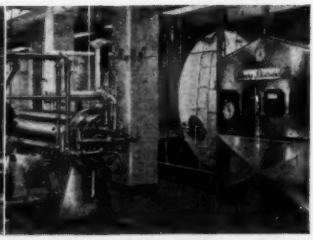

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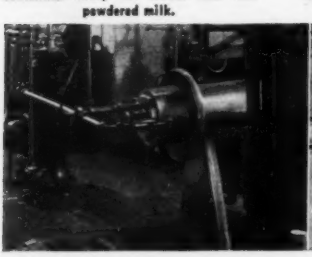

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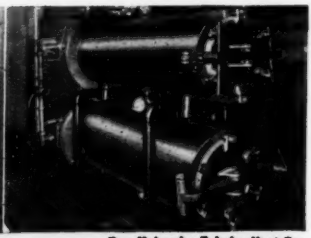

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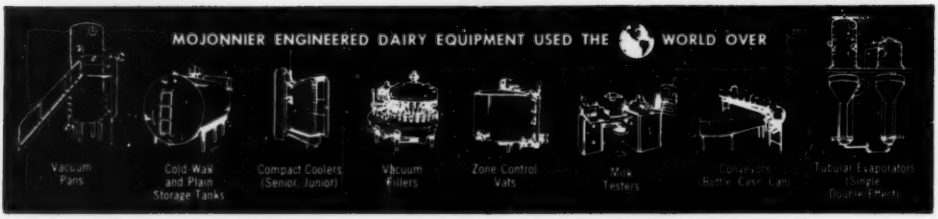

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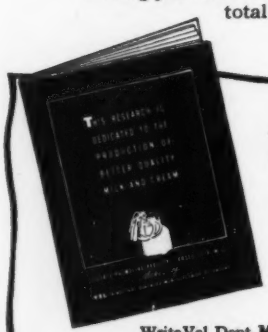

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Full account of VEL experiment

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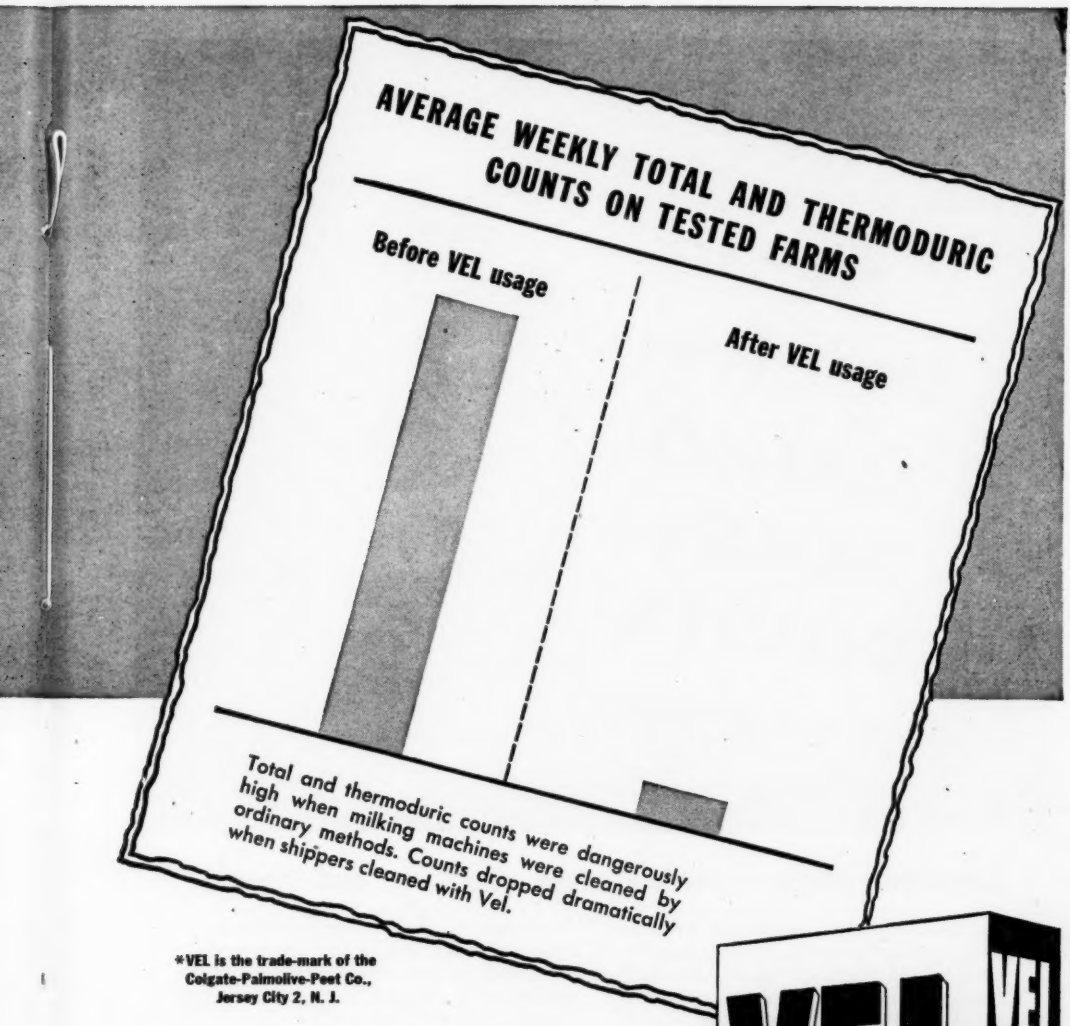
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Before VEL usage
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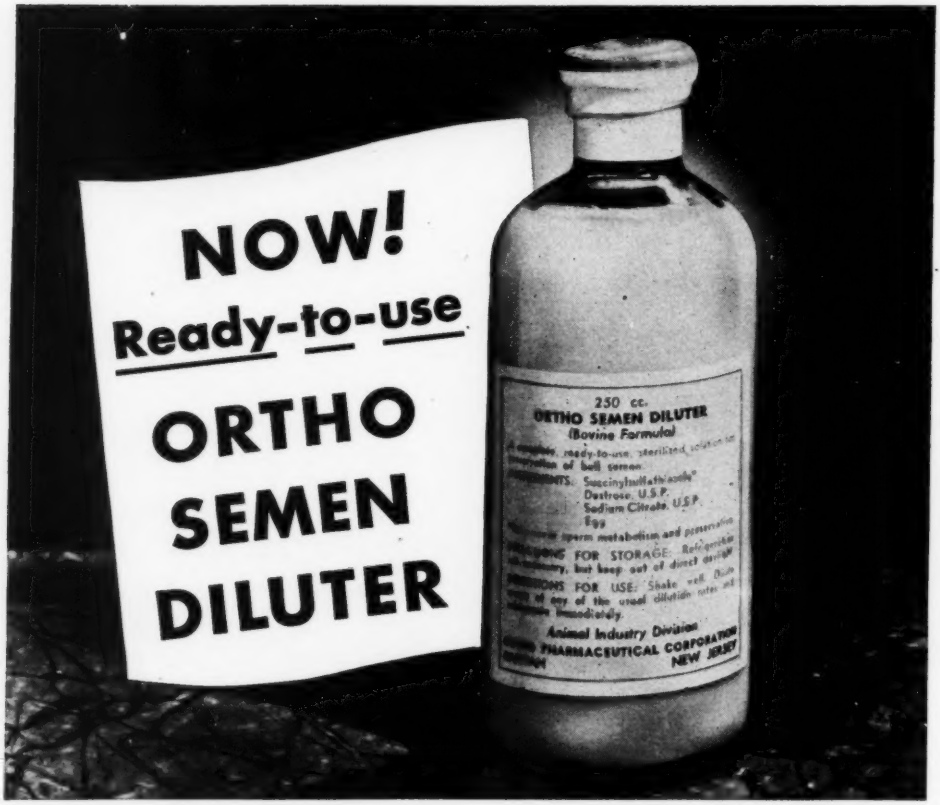

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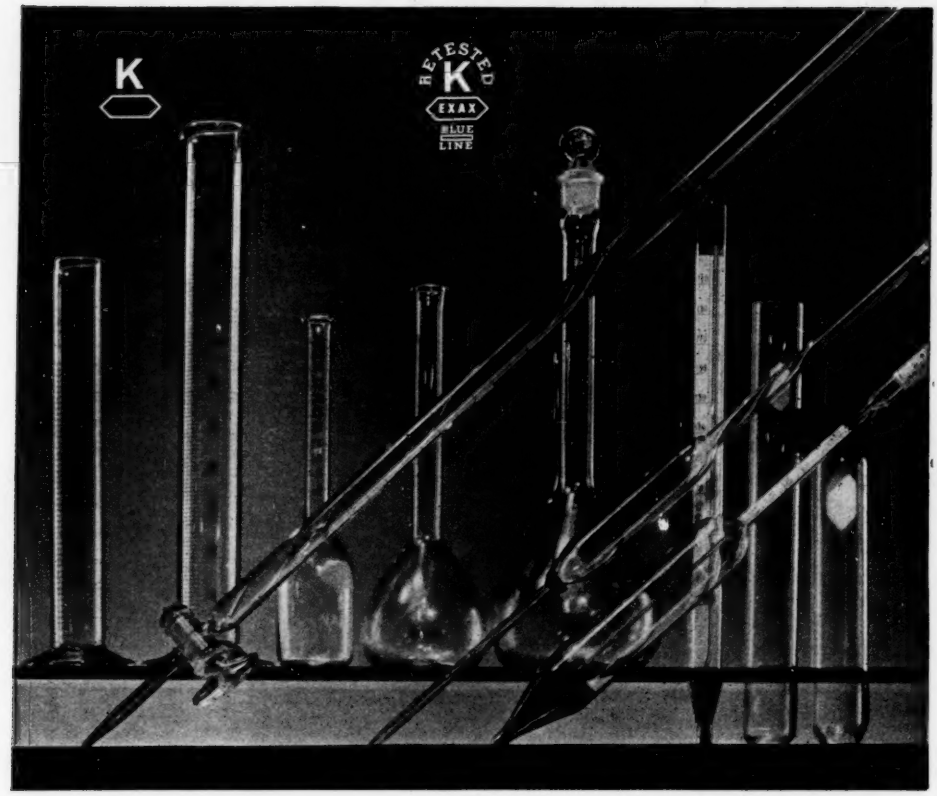

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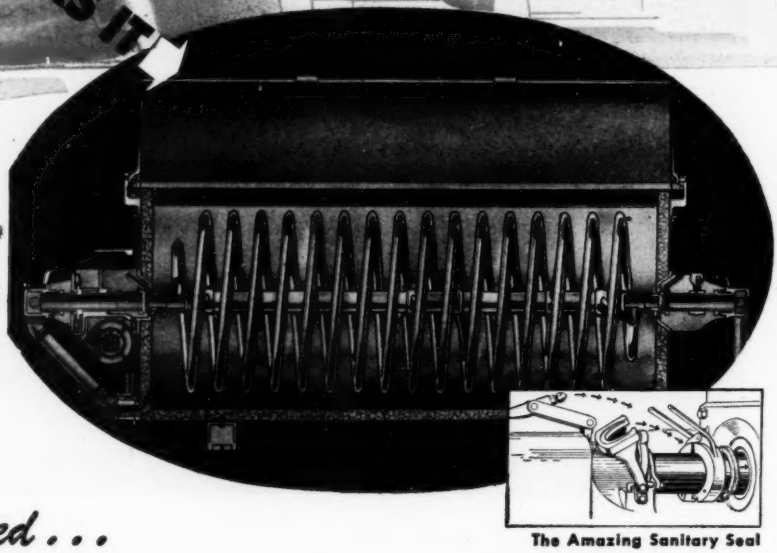

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JOURNAL OF DAIRY SCIENCE

VOLUME XXXI SEPTEMBER, 1948 NUMBER 9

THE EFFECT OF PYRIDIUM, PENICILLIN, FURACIN, AND PHENOXETHOL UPON THE LIVABILITY OF SPERMATOZOA AND UPON THE CONTROL OF BACTERIA IN DILUTED BULL SEMEN[1, 2]

R. H. FOOTE AND G. W. SALISBURY[3]

Laboratory of Animal Breeding and Artificial Insemination, Department of Animal Husbandry, Cornell University, Ithaca, New York

The same types of organisms associated with vaginal infections and abortion in cows sometimes are found in the semen of bulls. A majority of the sulfonamides tested on semen samples (7) markedly reduced bacterial growth at levels which also increased the livability of the spermatozoa. Whether or not this improvement in spermatozoan livability which accompanied bacteriological control was a phenomenon distinctly associated with the sulfonamide compounds was not established.

The bacteriostatic and/or bactericidal effects of pyridium (8), penicillin (2), furacin (4, 5), and phenoxethol (3, 9) have been demonstrated on a wide variety of organisms. Almquist *et al.* (1) reported that penicillin controlled bacterial growth in diluted bull semen at levels which had no significant effect on livability or fertility. Phillips and Lardy (11) noted that "certain antibiotics, including penicillin, were not harmful to sperm motility". So far as is known to the authors, no studies have been reported on the effects of adding pyridium, furacin, and phenoxethol to diluted bull semen. The present paper is a report of the comparative effect of pyridium, furacin, phenoxethol, two penicillins, and sulfanilamide on bacterial growth and spermatozoan livability.

EXPERIMENTAL

The design and methods used in conducting the experiments reported in this paper were the same as those described previously (7). Solutions

Received for publication February 18, 1948.

[1] The data published in this paper have been taken from a thesis presented by the senior author to the Graduate School, Cornell University, in partial fulfillment of the requirements for the degree of Master of Science in Agriculture, 1947.

[2] The authors are indebted to Merck and Company, Inc., for the pyridium; to the Eaton Laboratories, Inc., of the Norwich Pharmaceutical Company for the furacin; to the Heyden Chemical Corporation for one of the commercial penicillins, and to A. H. Allard for technical assistance in these experiments.

[3] Now at the University of Illinois.

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of pyridium, furacin, and phenoxethol were prepared in the same manner as were the sulfonamide solutions (7). Since penicillin comes in sterile vials and is unstable to heat, varying concentrations of penicillin solutions were prepared aseptically just prior to use in each experiment.

Eighteen first ejaculates, 13 second ejaculates, and 2 third ejaculates were used in the experiment. These had an average initial motility of 66 per cent motile spermatozoa which were moving at an average rate of 3.2, where 4.0 is considered maximum. The mean concentration was 1,180,000 spermatozoa per mm.^{3}, and the mean methylene blue reduction time was 6.7 minutes.

Pyridium. The azo dye, pyridium, was soluble only to the extent of about 5 mg. per 100 ml. of citrate diluent. Apparently as a consequence of the low concentration, pyridium had very little effect on the livability of the spermatozoa or on the control of bacterial growth.

Five milligrams per 100 ml. of pyridium added to five semen samples stored in citrate diluent at 37.5° C. and eight semen samples stored in citrate-phosphate diluent at 20° C. had no observable effect, either beneficial or harmful, on the livability of the spermatozoa and failed to reduce bacterial growth as compared to the untreated controls.

Sulfanilamide, the positive control, was significantly superior statistically in maintaining livability of the spermatozoa. Also, it was highly effective in inhibiting bacterial growth.

Penicillin. Two commercial penicillins, *A* and *B*, each were added to four separate ejaculates stored at 20° C. in citrate-phosphate diluent at the rates of 0, 63, 125, 250, 375, and 500 Oxford Units of penicillin per ml. of diluted semen. All additions of penicillin *A* were toxic to the spermatozoa, whereas additions of penicillin *B* were not. Both penicillins were equally effective in reducing bacterial growth and were more effective than the 300 mg. per 100 ml. of sulfanilamide in this respect.

A subsequent experiment was conducted in yolk-citrate diluent at 5° C. By adding penicillin at the rate of 200, 400, 600 and 800 Oxford Units per ml. of diluted semen, penicillin *A* again was found to be toxic to the spermatozoa and penicillin *B* was not. The results are shown in table 1. As in the previous report (7), the mean motility values shown in the table were calculated from the individual observations made at specified intervals until the spermatozoa in a majority of the treatments were dead.

In maintaining the motility of the spermatozoa, sulfanilamide was found to be significantly superior, statistically, to all levels of penicillin tested. Where the treatments were not toxic, only sulfanilamide slowed the rate of motility. Whether commercial penicillin *A* was composed chiefly of a penicillin different from commercial penicillin *B* or whether it contained a substance which was toxic to the spermatozoa (12) was not determined.

In the ten samples used for the motility estimation, a study of bacterial growth after 4 and 8 days of storage revealed that bacterial contamination was small in all treatments. Neither the sulfanilamide nor the two penicillins were particularly effective in controlling the organisms which survived at 5° C. Countable plates were obtained from five of the seven ejaculates treated with penicillin *B*, the results of which are included in table 1. Similar results were recorded for penicillin *A*.

Furacin. It quickly was established that large quantities of furacin added to spermatozoa stored in the citrate-phosphate buffer at 20° C. were deleterious to the spermatozoa. Lower levels of furacin were studied on the semen obtained from four different bulls. At the four lowest levels

TABLE 1

The effect of additions of penicillin A and B to yolk-citrate upon the livability of spermatozoa and bacterial growth

Treatment	No. of ejaculates	Storage at 5° C.	Controls	Oxford units of penicillin/ml.				
			SA* 300 mg. %	0	200	400	600	800
		(*days*)	% motile spermatozoa					
Penicillin A	3	16	37.5	35.0	31.0	27.6	21.5	15.2
Penicillin B	7	16	42.4	39.4	40.0	39.4	39.1	36.3
			Rate of motility					
Penicillin A	3	16	1.5	1.6	1.1	0.9	0.7	0.4
Penicillin B	7	16	1.4	1.6	1.5	1.5	1.6	1.5
			Bacterial count per ml.					
Penicillin A	5	4	18,500	27,000	19,000	14,000	17,500	10,000
Penicillin B	5	8	14,000	25,000	16,000	11,500	13,000	10,000

* SA = Sulfanilamide.

of furacin studied (0, 0.5, 1.0 and 2.0 mg. per 100 ml. of diluent), the mean motilities for the experiment were 42, 41, 34 and 27 per cent, respectively, the last two means being significantly different, statistically, from the first two means. Therefore, furacin was considered to be spermicidal.

Furacin proved to be an effective bacterial antagonist at the higher concentrations, but levels which were not harmful to livability of the spermatozoa were only moderately effective in controlling bacterial growth. When furacin was added to two ejaculates at the rate of 0, 0.5, 1.0, 2.0, 5.0 and 10.0 mg. per 100 ml. of diluent, the average plate count after 72 hours of storage was 81,000,000, 24,800,000, 2,760,000, 106,000, 7,000 and 650 organisms per ml., respectively. The two controls containing 300 mg. per cent of sulfanilamide averaged 2,670,000 organisms per ml. of diluted semen.

TABLE 2

The effect of adding phenoxethol to citrate-phosphate diluent at 20° C. upon livability of spermatozoa and bacterial growth
(Mean of 3 ejaculates)

Agent	Conc. (%)	% motile	Rate of motility	Bacterial Count/ml.[a]
Phenoxethol	0	52.0	2.6	44,000
	0.125	20.0	0.6	8,000
	0.25	0.0	0.0	2,000
	0.50	0.0	0.0	1,500
	1.00	0.0	0.0	900
	2.00	0.0	0.0	100
Sulfanilamide	300 mg.%	53.3	2.2	22,000

[a] Platings made after 24 hr.

Phenoxethol. None of the compounds previously mentioned exerted any marked bacteriostatic effect on *Pseudomonas pyocyaneus.* The reports in the literature (3, 9) that phenoxethol was effective against this organism were substantiated in this laboratory using a pure culture of pseudomonas organisms isolated from a sample of semen.

Varying concentrations of phenoxethol were added to three semen samples and were found to be highly spermicidal. In the same study, after 24 hours of storage, subsamples were taken for plating. The data are presented in table 2. While all levels of phenoxethol tested partially inhibited bacterial growth. *Pseudomonas pyocyaneus* continued to thrive at the 0.125 and 0.25 per cent levels of this drug.

Numbers of bacteria present in fresh semen. Throughout the course of these studies sufficient data were accumulated to make possible a comparative study of the numbers of bacteria present in the first and second ejaculates collected within a few minutes of each other. The data for 47 such comparisons are summarized in table 3.

TABLE 3

A comparison of the number of organisms found in first and second ejaculates collected from bulls of high and low fertility

Bulls	Ejaculates	Range of bacteria in thousands per ml.										
		1–4	5–9	10–24	25–49	50–99	100–249	250–499	500–999	1,000–2,499	2,500–4,999	Over 5,000
		No. of ejaculates in each group										
NYABC[a]	1st	2	4	3	3	6	4	2	0	1[b]	0	1[b]
	2nd	4	3	3	6	4	4	0	1[b]	1[b]	0	0
Vitamin A deficient	1st	1	0	0	0	0	4	3	7	4	2	0
	2nd	1	0	0	0	1	3	9	4	3	0	0

[a] NYABC = New York Artificial Breeders Cooperative, Inc.
[b] Bulls of low fertility.

Twenty-four of the 26 bulls used by the New York Artificial Breeders Cooperative, Inc., were highly fertile during the period in which the collections were made. The other two bulls were disposed of shortly thereafter because of their low fertility records. The vitamin A-deficient bulls, from which 21 paired ejaculates were collected, were not used for breeding purposes. However, they were believed to be of relatively low fertility. These results are in agreement with Gunsalus *et al.* (10), who found more bacteria in the first than in subsequent ejaculates. Futhermore, the semen from bulls of known high fertility contained fewer organisms than semen from the other bulls studied.

DISCUSSION

The data presented in this paper show that a number of compounds, chemically unrelated to the sulfonamides, effectively controlled bacterial growth. With the exception of penicillin, it was impossible to establish drug levels which were consistent with bacterial control and at the same time were not detrimental to the spermatozoa. This is in rather sharp contrast with the previous report (7) in which nine out of 12 sulfonamides in bacteriostatic or bactericidal concentrations exerted a favorable influence upon the livability of the sperm cells. This favorable influence appears to be associated with the "slowing down" of the spermatozoa noted when certain sulfonamides were present in the diluent. These phenomena were not observed when the compounds reported herein were added to the different diluents.

Penicillin, while exerting no apparent beneficial effect on the spermatozoa, was slightly superior to sulfanilamide in controlling bacterial growth. However, the decided differences in toxicity observed between the two penicillins studied present a practical problem should it become desirable to take advantage of the bactericidal properties of penicillin. The use of synthetic penicillins (6) which recently have become available offers a possible approach to the problem.

SUMMARY AND CONCLUSIONS

1. The possible usefulness of pyridium was limited by its low solubility. Maximum attainable concentrations produced no noticeable effects on spermatozoan livability or on bacterial growth.

2. Two commercial penicillins were equally effective in controlling bacterial growth and were slightly superior to sulfanilamide in this respect. Neither penicillin proved to be beneficial to the spermatozoa, and one in particular was toxic even when present in small amounts.

3. Furacin and phenoxethol proved to be highly bactericidal, and at the same time were spermicidal at most of the levels tested. Organisms of the pseudomonas group, especially, were resistant to the lower concentrations studied.

4. Throughout these experiments only sulfanilamide, the positive control, actually improved the livability of the spermatozoa.

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THE EFFECT OF SULFONAMIDES UPON THE LIVABILITY OF SPERMATOZOA AND UPON THE CONTROL OF BACTERIA IN DILUTED BULL SEMEN[1]

R. H. FOOTE AND G. W. SALISBURY[2]

Laboratory of Animal Breeding and Artificial Insemination, Department of Animal Husbandry, Cornell University, Ithaca, New York

The possible influence of bacteria on the results of metabolic studies and on the results of fertility in artificial insemination has been under investigation at this laboratory for some time. With the present trend of shipping the diluted semen over greater distances and inseminating animals after the diluted semen has undergone longer periods of storage, other factors, in addition to the original quality, may become increasingly important in maintaining high levels of fertility. One such factor is bacteriological control.

In view of the fact (6) that sulfonamides, with proper variation of the factors influencing sulfonamide action, inhibit the metabolism of cells of nearly every variety, and that certain sulfonamides increased the livability (8, 12) and fertility (13) of bovine spermatozoa, several sulfonamides were selected for study. Furthermore, contrary to earlier findings (3, 7, 10, 17), several investigators (5, 15, 18) reported that sulfonamides administered in human therapy did not impair the livability of the spermatozoa. Likewise *in vivo* and *in vitro* studies with rams (1) and rodents (4, 9, 11, 14) failed to demonstrate that the sulfonamides were deleterious to spermatogenesis or to the survival of the spermatozoa. Since levels of sulfonamides consistent with bacteriological control and optimum for the survival of the sperm cells had been ascertained for sulfanilamide only (8), it was logical that investigations were undertaken to study various sulfonamides in these respects.

EXPERIMENTAL

Materials and methods. Because of the large number of sulfonamides tested and the limited quantity of semen in each ejaculate, it was impossible to compare the sulfonamides with each other by adding all of them to the same series of ejaculates. Therefore, each experiment included a positive control containing sulfanilamide at the established optimum level of 300 mg. of sulfanilamide per 100 ml. of diluted semen (8), and a negative control

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[1] The data published in this paper have been taken from a thesis presented by the senior author to the Graduate School, Cornell University, in partial fulfillment of the requirements for the degree of Master of Science in Agriculture, 1947.

[2] Now at the University of Illinois.

containing no sulfonamide. By means of these positive and negative controls it was possible to compare the relative effects of each treatment employed.

Since length of life in storage was considered the best measure of the effect of each sulfonamide on livability of the spermatozoa, microscopic examinations to determine the percentage of motile spermatozoa and the rate of motility were made until the spermatozoa in a majority of the treatments were dead. The motility values recorded at each observation were used in calculating the mean motility values presented in the accompanying tables. Tubes containing the stored samples were coded, and at the time of each microscopic examination they were randomized to prevent the introduction of bias on the part of the investigator due to knowledge of the treatment in the particular sample under observation.

Three series of experiments were conducted. In the series I experiments, bacterial growth was measured by changes in optical density of the stored semen samples as described by Knodt and Salisbury (8) and by the plate count method. Thereafter, only the plate count method was used, for which veal infusion agar served as the culture medium. Plates were counted after 48 and 96 hours of incubation at 37.5° C. Only the total counts recorded at 96 hours are presented in this paper, inasmuch as colonies of the slow-growing diphtheroids frequently were too small to count at 48 hours.

The sulfonamides, or sulfas as they sometimes are called, selected for use were sulfathiazole (ST), sulfapyridine (SP), sulfamerazine (SM), sulfaguanidine (SG), sulfaquinoxaline (SQX), sodium sulfadiazine (NaSD), sodium sulfathiazole (NaST), carboxysulfathiazole (Carb.ST), sodium sulfamerazine (NaSM), sodium sulfamethazine (NaSMT), N^1-benzoylsulfanilamide (N^1-BSA), sulfasuxidine (SS), and sulfanilamide (SA) as a control.

All sulfonamide solutions were prepared by adding the desired quantities of each sulfonamide to the basic diluter used. These solutions then were autoclaved simultaneously at 15 lb. pressure for 20 minutes.

The 23 first ejaculates, 11 second ejaculates, and 2 third ejaculates used had a mean initial motility of 66 per cent motile spermatozoa moving at a rate of 3.3, where 4.0 is considered maximum, a mean concentration of 1,069,000 spermatozoa per $mm.^3$, and a mean methylene blue reduction time of 6.9 minutes.

RESULTS

Series I. Semen diluted with citrate-sulfonamide diluter and stored at 37.5° C. In this series of experiments the basic diluter consisted of 3.6 g. of sodium citrate dihydrate made up to 100 ml. of water distilled in glass. One part of fresh semen was added to nine parts of the citrate-sul-

fonamide diluter and stored at 37.5° C. Six sulfonamides, at 0, 50, and 100 per cent of the maximum solubility of each drug as established for this diluter, were studied initially. Motility and optical density observations were made at 0, 6, 12, 24, and 48 hours of storage, and subsamples were taken for bacterial counts at 24 and 48 hours of storage.

After five ejaculates had been so treated, this study was terminated, for in nearly all cases most of the spermatozoa were dead after less than 12 hours of storage. Because of the adverse effect at 37.5° C. of the diluting medium upon the livability of the spermatozoa, it was impossible to measure any effect that the particular sulfonamides tested may have had on the livability of the spermatozoa.

The optical density of the stored samples increased appreciably. However, the formation of the sulfonamide crystals in several of the treatments as the acidity increased in the stored samples interfered with the measurement of optical density changes associated with bacterial growth. Consequently only bacterial growth as measured by the plate count method will be reported. These data are summarized in table 1. The drugs are ranked on the basis of the effectiveness with which each controlled bacterial growth. Sulfanilamide, as well as several other sulfonamides, exerted a pronounced bacteriostatic effect. As a rule, bacterial growth was inversely proportional to the amount of the sulfonamide present, irrespective of the particular one used.

TABLE 1

Bacterial growth in diluted semen stored with different sulfonamides at 37.5° C. as measured by plate counts
(Mean of 3 ejaculates)

Drug	Conc.	Rank at 24 hr.	Plate counts (1,000's/ml.) after storage for:		Rank at 48 hr.
			24 hr.	48 hr.	
	(mg. %)				
SA	300	1	252	18,300	2
ST	200	2	401	13,000	1
ST	100	3	9,170	26,200	3
SA	150	4	10,300	68,500	5
SM	90	5	20,200	64,000	4
SG	90	6	34,400	144,000	8
SQX	50	7	40,600	139,000	7
SP	100	8	50,700	97,000	6
SQX	25	9	66,800	178,000	9
SG	45	10	72,800	323,000	12
SM	45	11	80,100	197,000	10
SP	50	12	102,000	221,000	11
O	.....	13	793,000	4,970,000	13

Series II. Semen diluted with citrate-phosphate-sulfonamide diluter and stored at 20° C. The poor livability encountered in the experiments

reported in series I was circumvented by reducing the temperature to 20° C. and by developing a more suitable diluter. The new diluter, C-P, consisting of 0.2 g. potassium dihydrogen phosphate, 0.29 g. anhydrous disodium phosphate, and 1.8 g. of sodium citrate dihydrate made up to 100 ml. water distilled in glass, was found to preserve the motility of spermatozoa for several days at 20° C. The pH of this diluent, following autoclaving, was

TABLE 2

The effect of sulfonamide additions to C-P diluter upon the percentage of motile spermatozoa
(Means for the entire experiment)

No. of ejaculates	Name of drug	Maximum sol. of each drug	% motile spermatozoa					
			Levels of the drugs expressed as % of maximum solubility				Controls	
			12.5	25	50	100	0	SA 300 mg. %
		(mg. %)						
3	ST	200	.....	37.7	43.3	24.4	37.0	45.0
5	ST	.....	.....	27.4	33.2	Toxic	29.8	33.0
8	ST	.....	.....	.....	36.6	Toxic	33.7	35.8
3	SP	100	.....	37.7	32.0	27.7	37.0	45.0
5	SP	.....	.....	29.0	23.6	Toxic	29.8	33.0
8	SP	.....	.....	.....	27.9	Toxic	33.7	35.8
3	SM	90	.....	44.1	40.7	37.8	37.0	45.0
5	SM	.....	.....	32.0	28.7	N.T.[a]	29.8	33.0
8	SM	.....	.....	.....	32.1	N.T.	33.7	35.8
3	SG	90	.....	44.7	40.7	37.8	37.0	45.0
5	SG	.....	.....	29.2	31.0	N.T.	29.8	33.0
8	SG	.....	.....	.....	34.2	N.T.	33.7	35.8
3	SQX	50	.....	40.7	40.1	42.7	37.0	45.0
5	SQX	.....	.....	29.7	31.4	N.T.	29.8	33.0
8	SQX	.....	.....	.....	34.4	N.T.	33.7	35.8
9	NaSD	250	.....	34.4	34.6	31.2	30.8	33.3
3	NaST	500	.....	17.1	13.4	9.1	23.1	22.3
4	NaST	.....	34.0	34.0	28.8	Toxic	37.5	37.8
6	NaST	.....	...	24.9	Toxic	Toxic	31.2	31.3
4	Carb.ST	600[b]	28.8	31.8	27.1	20.5	27.3	27.0
4	NaSM	500	.....	29.8	26.8	Crystals	27.3	27.0
5	NaSMT	500	.....	31.8	36.2	Crystals	30.6	28.8
4	N[1]-BSA	500[b]	.....	26.0	22.8	5.6	26.8	28.8
4	SS	600[b]	25.8	25.0	25.8	19.8	27.3	27.0

[a] N.T. = not tested as previous tests indicated toxicity.
[b] arbitrary maximum as these drugs were highly soluble.

6.90 as compared to the autoclaved citrate used previously which had a pH of about 7.75.

Motility observations were made on the semen samples at 8, 24 and 48 hours, and every 24 hours of storage thereafter, until most of the spermatozoa were dead. Usually progressive movement was observed in the diluted semen samples for at least 6 days under these conditions. In other respects, these experiments were conducted in the same manner as were those in series I.

The percentage of motile spermatozoa and the rate of motility were observed on the stored ejaculates from 15 bulls. Since it was found that with two exceptions the relative rates of motility paralleled the relative percentages of motile spermatozoa, "rate" observations will be discussed only briefly. Means for the percentage of motile spermatozoa are shown in table 2.

At the higher concentrations (100 per cent of maximum solubilities of each drug), all of the more soluble sulfonamides were toxic. When a particular level of a sulfa was established as toxic or at least not beneficial to the spermatozoa, lower concentrations of the drug were studied. These

TABLE 3

Bacterial growth in diluted semen stored with different sulfonamides at 20° C. as measured by plate counts

(Mean of 4 ejaculates; platings made after 24 hr. of storage)

Name of drug	Drug conc.	Rank	Plate counts (1,000's/ml.)
	(mg. %)		
ST	100	1	36[a]
SA	300	2	52
SA	150	3	99
ST	50	4	163
NaSD	500[b]	5	200
NaSD	250[b]	6	252
SP	50	7	300
SP	25	8	309
SM	45	9	313
SM	22.5	10	334
SQX	25	11	417
SQX	12.5	12	547
SG	45	13	570
SG	22.5	14	1,637
O	.....	15	5,900

[a] Some colonies hidden by spreading organisms.
[b] Sulfa crystals observed.

results are included in the table. Nine of the 12 drugs tested were superior to the negative controls in preserving the life of the spermatozoa, while three of the 12 drugs equaled or excelled sulfanilamide in this respect. Of these three—sodium sulfadiazine, sodium sulfamethazine, and carboxysulfathiazole—only the latter two were significantly superior to sulfanilamide (significant, respectively, at the 5.0 and 1.0 per cent levels of probability). The apparent superiority of sodium sulfamethazine and carboxysulfathiazole over sulfanilamide may be due to the fact that for some unknown reason sulfanilamide in the control failed to benefit the spermatozoa.

The additions of sulfanilamide consistently decreased the rate of movement of the spermatozoa. While the difference between the negative and positive controls was not great, it was significant statistically. N^1-benzoyl-

sulfanilamide markedly reduced the rate of movement and almost completely immobilized the spermatozoa long before they died. No particular pattern was established by the other sulfonamides.

Plate counts were made on all semen samples stored for the motility observations, but several of the samples contained large numbers of spreading organisms which prevented accurate counting. The number of bacteria in the stored semen samples from which countable plates were obtained is reported in tables 3 and 4. As was expected, the organisms

TABLE 4

Bacterial growth in diluted semen stored with different sulfonamides at 20° C. as measured by plate counts

(Mean of 2 ejaculates; platings made after 24 hr. of storage)

Name of drug	Drug conc.	Rank	Plate counts (1,000's/ml.)
	(mg. %)		
N1–BSA	500	1	13
SA	300	2	15
NaST	250	3	20
N1–BSA	250	4	31
NaST	500	5	33
Carb.ST	150	6	38
N1–BSA	125	6	38
NaST	125	8	40
NaSMT	500[a]	9	46
NaSMT	125	10	51
NaSM	500[a]	11	58
NaSM	250	12	64
SS	300	13	81
NaSMT	250	14	97
Carb.ST	300	15	103
NaSM	125	16	114
Carb.ST	75	17	169
SS	75	18	176
SS	150	19	255
O	.....	20	303

[a] Sulfa crystals observed.

multiplied rapidly at the storage temperature of 20° C. in the negative controls, although much less rapidly than at 37.5° C. Here again several sulfonamides markedly inhibited bacterial growth. Increasing the concentrations of the sodium salts of several sulfonamides beyond the point where crystallization occurred did not increase materially the bacteriostatic effect of the drugs. In one experiment, 0.25, 0.50, 1.0, 2.0, 4.0, 6.0, and 8.0 g., respectively, of NaSD per 100 ml. were added to diluted semen. Additions beyond the saturation point (0.25 g.) did not increase the effectiveness with which bacteria were controlled.

Series III. Semen diluted with yolk-citrate-sulfonamide diluter and stored at 5° C. Under routine conditions in artificial insemination, buffered egg yolk is used as the diluent, and the diluted semen is stored at 5° C.

Therefore, it was desirable to test, under these conditions, concentrations of the drugs which reduced bacterial growth and/or improved spermatozoan livability in the citrate-phosphate diluter at 20° C. Sulfathiazole, NaSD, NaSMT, and Carb.ST were shown to be equal or superior to sulfanilamide in at least one of these respects.

To accommodate the four drugs tested at four different levels of each drug plus a positive and negative control for each drug, five semen samples were split into 24 equal subsamples. One part of semen was added to nine parts of diluter. Motility examinations were made every 2 days until a majority of the treatments showed no motile spermatozoa. The mean

TABLE 5

The effect of ST, NaSD, NaSMT, and Carb.ST upon the livability of spermatozoa in yolk-citrate stored at 5° C.

(Mean of 5 ejaculates, and a total of 1200 observations)

Drug tested						Controls	
						No sulfa	SA 300 mg. %
ST	mg. % in diluter	50	75	100	125	.....	.....
	% motile sperm.	36.4	35.3	33.9	30.6	35.0	37.6
NaSD	mg. % in diluter	150	200	250	300	.....	.....
	% motile sperm.	33.7	32.4	33.0	31.9	35.8	37.3
NaSMT	mg. % in diluter	100	150	200	250	.....	.....
	% motile sperm.	33.1	33.4	29.9	26.9	35.6	38.0
Carb.ST	mg. % in diluter	50	100	150	200	.....	.....
	% motile sperm.	39.0	40.4	36.6	31.8	36.1	38.3

motility for each of the treatments is presented in table 5. Appropriate statistical analyses revealed that the differences observed between the negative control and both carboxysulfathiazole and sulfanilamide were significant at the 5 per cent level of probability.

More than 1,000 plate counts[3] were made after 4 and 8 days of storage to determine the number of organisms present in the diluted semen used for the motility observations. Most of the organisms in the five ejaculates studied did not live well at the storage temperature of 5° C. Consequently, little differentiation due to treatment was observed. At 4 days the counts

[3] By washing the eggs according to Bryant and Sharp (2), wearing sterilized rubber gloves, and using all sterilized equipment, it was possible to obtain the egg yolk used in the diluent free, or almost completely free, from bacteria.

ranged from 11,000 to 43,000 organisms per ml. and at 8 days from 13,800 to 109,000 organisms per ml., with the low count being obtained from a semen sample to which sulfanilamide had been added and the high count from a sample to which no bacterial inhibitor had been added. Standard laboratory tests for the identification of *Pseudomonas pyocyaneus* showed a small percentage of pseudomonas organisms in the fresh semen of three out of the five ejaculates. After 4 and 8 days of storage, the pseudomonas organisms made up a large proportion of the total number of organisms present.

DISCUSSION

The experimental evidence presented in this paper demonstrates that all of the 12 sulfonamide preparations investigated were effective in reducing the rate of bacterial growth. Plate counts made on diluted semen stored for 24 hours at 20 and at 37.5° C. showed that sulfanilamide, sulfathiazole, and some of the other sulfonamides generally prevented the bacterial population from increasing to more than about four times that at zero time. The bacterial population increased 800 times over the population at zero time in diluted semen containing no sulfonamide.

At 5° C. and in the presence of egg yolk, none of the sulfonamides tested markedly decreased the growth of the organisms which were able to survive the storage temperature of 5° C. A majority of these surviving organisms were of the pseudomonas group. At the temperatures studied, the pseudomonas group was not inhibited markedly by levels of the sulfonamides which were not harmful to the spermatozoa.

The evidence presented shows that large doses of the sulfonamides may be toxic to the spermatozoa. However, with three exceptions, levels were established for each sulfonamide which improved the livability of the spermatozoa and, at the same time, were consistent with bacteriological control. Even at 20° C., satisfactory motility of the spermatozoa was maintained for many days in the presence of several of the sulfonamides.

While sulfanilamide fairly consistently increased the duration of motility, it consistently decreased the rate of motility. In view of the report of Knodt and Salisbury (8) that oxygen consumption by spermatozoa is depressed in the presence of sulfanilamide, this slowing down of the spermatozoa is believed to result from a depressing effect by sulfanilamide on cellular metabolism.

SUMMARY AND CONCLUSIONS

1. Twelve sulfonamides were added to bull semen diluted with citrate-phosphate and stored at 20° C. At the level of each drug determined to be optimum for spermatozoan survival, nine of the 12 drugs increased the livability of the spermatozoa over that observed when no sulfonamide was

added to the diluent. Of these nine, only two sulfonamides, sodium sulfamethazine and carboxysulfathiazole, were significantly superior to sulfanilamide in maintaining motility of the sperm cells, but they were inferior in controlling bacterial growth. Sulfanilmide slightly decreased the rate of motility, and N^1-benzoylsulfanilamide exerted a similar but more pronounced effect. No consistent pattern was established by the other sulfonamides.

2. At 20 and at 37.5° C. all of the sulfonamides were effective in reducing bacterial growth at levels which were not harmful to the spermatozoa. *Pseudomonas pyocyaneus* was not inhibited at these levels.

3. At 5° C. the sulfonamides were only slightly effective in controlling bacterial growth because the pseudomonas group of organisms predominated in the bacterial flora surviving at this temperature.

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BREEDING BEHAVIOR, SPERMATOGENESIS, AND SEMEN PRODUCTION OF MATURE DAIRY BULLS FED RATIONS LOW IN CAROTENE

R. W. BRATTON, G. W. SALISBURY,[1] T. TANABE,[2] C. BRANTON,[3] E. MERCIER,[4] AND J. K. LOOSLI

Department of Animal Husbandry, Cornell University, Ithaca, New York

The present emphasis on extending the use of the desirably proved dairy sire in artificial breeding has focused attention on the need for more information concerning the nutrition of the mature sire, particularly with respect to the role of nutrition in semen production and fertility. In both, spermatogenesis is of primary concern.

The importance of vitamin A and carotene in developing and maintaining the normal germinal epithelium and the breeding ability of young bulls has been demonstrated by Sutton *et al.* (14), Jones *et al.* (7), Hodgson *et al.* (6) and Erb *et al.* (4, 5). Regeneration of the germinal epithelium following vitamin A and carotene therapy has been observed (4, 6). Similar studies with mature bulls have not been reported.

Roughages are the principal source of carotene for bulls of breeding age. If allowed pasture, mature bulls might be expected to build up body stores of vitamin A sufficient to carry them through periods of relatively low carotene intakes. On the other hand, the continued use of low-carotene roughages might deplete body stores to the extent that breeding ability and spermatogenic activity would be impaired. This possibility raises the question as to how long mature bulls will continue to produce normal semen and remain free from clinical manifestations of vitamin A deficiency when fed a low-carotene ration of concentrates and poor quality roughage. An investigation was undertaken in August, 1945, to measure the changes in semen production and to study the development of clinical manifestations of vitamin A deficiency in mature dairy bulls.

EXPERIMENTAL PROCEDURE

The experimental plan was to feed six relatively mature breeding bulls a low-carotene concentrate mixture along with poor quality roughage for a period of at least 1 year, unless serious clinical symptoms developed earlier. No deficiencies were apparent by the end of that period of time. Therefore, the roughage component of the ration was changed to dried beet pulp,

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[1] Now at the University of Illinois.

[2] Now at the University of Wisconsin.

[3] Now at the Louisiana State University.

[4] Now at the Ministry of Agriculture, Quebec, Canada.

and three of the bulls were fed a supplement of carotene in oil, sufficient to furnish approximately 30 mg. of carotene per 100 lb. of body weight per day. The remaining three bulls received no supplement of carotene. The 30-mg. level of carotene supplementation represented approximately 5.5 times the level for maintenance of beef bulls of equivalent weight as recommended by the Committee on Animal Nutrition of the National Research Council (11).

It was not possible at the beginning of the experiment in August, 1945, to secure six bulls that were over 5 years of age. However, semen had been collected from the two youngest bulls at irregular intervals for approximately 1 year before the initiation of the experiment. The breed and age of the bulls at the beginning of the experiment were as follows: *N*, a Holstein, age 4 years, 7 months; *C*, a Brown Swiss, age 2 years, 10 months; *J*, a Guernsey, age 8 years, 6 months; *M*, a Guernsey, age 6 years, 4 months; *K*, a Guernsey, age 8 years, 7 months; *I*, a Guernsey, age 2 years, 9 months.

TABLE 1

Carotene content of the roughages fed

Periods during which a particular roughage was fed	Kind of roughage	Carotene content
		(*mg. /lb.*)
1 to 6, incl.	Hay	4.24
7 to 10, incl.	Hay	1.27
11	Hay	1.70
12	Wheat straw	1.70
13	Wheat and oat straw	0.56
14 to 16, incl.	Oat straw	1.80
17 to 21, incl.	Dried beet pulp	..... [a]

[a] Not analyzed; assumed to have no carotene or vitamin A potency.

The feed allowance for individual bulls was established on the basis of their individual initial body weights, their appetites and their general tendency to lose or gain in body weight. Changes in the feeding schedule were infrequent. Roughage feeding was scheduled to supply approximately 60 per cent of the T.D.N. and the concentrate mixture the remaining 40 per cent.

The concentrate mixture fed consisted of 40 lb. of ground oats, 10 lb. of ground barley, 12 lb. of ground wheat, 17 lb. of wheat bran, 8 lb. of soybean oil meal, 4 lb. of linseed oil meal, 7 lb. of cane molasses, 1 lb. of steamed bone meal and 1 lb. of common salt. This mixture analyzed 0.22 mg. of carotene per pound. The calculated digestible protein content was 13.8 per cent and the T.D.N. content 71.8 per cent.

The carotene content of the roughages fed and the daily feed intakes of the bulls are shown in tables 1 and 2, respectively.

The response criteria and the methods for their measurement were as follows:

Body weights. In addition to initial and final weights on each bull, monthly average weights were secured representing one, two or three daily weights, all of which were taken within a 7-day interval, usually at the beginning of the monthly period.

Blood plasma carotene and vitamin A. The carotene and vitamin A content of blood plasma from each bull was determined by the procedure of Kimble (8) on samples obtained on 3 successive days each month.

Semen production. From each bull two ejaculates were collected at 10-day intervals by means of an artificial vagina. The volume of semen per ejaculate, per cent motile spermatozoa, total spermatozoa per milliliter of semen and the per cent abnormal spermatozoa in each ejaculate were measured according to the standard procedures of this laboratory (10, 13, 15).

TABLE 2

Daily feed intakes of the bulls by periods

Periods	Kind of feed	lb. per day fed each bull					
		Bull *N*	Bull *C*	Bull *J*	Bull *M*	Bull *K*	Bull *I*
1 to 7, incl.	Concentrates	8	8	8	8	8	8
	Hay	18	18	18	18	18	12
8 to 16, incl.	Concentrates	8	8	8	7	8	8
	Hay or straw	18	18	18	14	18	12
17 to 21, incl.	Concentrates	8.4	8.2	6.2	6.2[a]	7.2	6.2
	Dried beet pulp	12.4	12.0	9.2	9.2	10.8	9.2
	Carotene[b] (mg.)	(576)	(0)	(404)	(0)	(476)	(0)

[a] During period 21, the scheduled amounts for bull *M* were reduced one-half.
[b] Carotene in oil, analyzing 35,371γ per g. of oil, was fed daily to furnish the intakes indicated. Values in parentheses are mg. per bull per day.

Clinical manifestations of vitamin A deficiency. The time intervals, in days, between the beginning of the feeding of the dried beet pulp, with and without the carotene-in-oil supplement, and the onset of night blindness, papillary edema, incoordination, edema of the extremities, loss of libido and breeding ability were noted.

Histological examination of testes. Testis tissue, obtained at the time the bulls were slaughtered, was fixed in Allen's PFA_3 fluid (modified Bowin's) (9), within 30 to 40 minutes after death. Microscopic examination of the seminiferous tubules for the extent of degeneration of the germinal epithelium was made on 7-μ sections which had been stained with an iodine-ripened hematoxylin (2) and counterstained with eosin.

Liver carotene and vitamin A. The whole livers were removed at death, stored at 40° F. and ground and sampled within 12 hours after death. A 10- to 25-g. composite sample was used for the extraction and determination of carotene and vitamin A according to the method of Davies (3).

Feed analyses. Chemical analyses of samples of the concentrates and roughages fed were made according to the methods of the A.O.A.C. (1). Carotene in the feeds as fed was estimated by the method of Nelson *et al.* (12).

The carotene content of the "carotene in oil" was determined colorimetrically by dissolving the oil in petroleum ether and measuring the density of the yellow color in a Lumitron colorimeter with a 440 mμ filter, the colorimeter previously having been standardized against pure β-carotene.

Analysis of data. In order to summarize the voluminous observations made during the experiment, the data were consolidated into twenty-one 30-day periods, each period representing three 10-day semen collection intervals. Within each of the 30-day periods, the observations made of each of the following criteria—body weights, blood plasma carotene and vitamin A, volume of semen per ejaculate, per cent motile spermatozoa, total spermatozoa per milliliter of semen and per cent abnormal spermatozoa per ejaculate—were summed and the average obtained. These averages then were considered as single observations in all subsequent analyses of the data. All semen data averages were based on the combined first and second ejaculates. All bulls finished the first 16 periods but during the following 5 periods one pair of bulls, *K* and *I*, was slaughtered because of the development of severe avitaminosis A by bull *I*.

RESULTS AND DISCUSSION

The 30-day period averages for body weight, estimated daily intakes of carotene, and plasma carotene and vitamin A of each bull are presented in tables 3 and 4. During the first 16 periods (480 days), the bulls generally increased in body weight (table 3). During the final 5 periods (150 days) two of the bulls, *M* and *I*, which were not receiving the carotene supplement, showed slight losses in body weight with the onset of avitaminosis A. In contrast, bull *C* gained in weight during these latter periods.

In table 4 the plasma carotene and vitamin A values reveal some apparent deviations from a smooth curve, but, in general, these variations can be explained by the carotene intakes. The plasma vitamin A level was low (2 to 3 γ per 100 ml. plasma) for bulls *C* and *M* before pronounced deficiency symptoms were evident.

The averages for each bull, by 30-day periods, for the criteria of semen production and spermatozoan characteristics are shown in tables 5 and 6. No consistent changes in relative semen volume were demonstrated during the first 16 months of the experiment (table 5). The per cent of motile spermatozoa (table 6) showed marked decreases for bulls *C* and *I* during the 11th, 12th, and 13th periods. These decreases in motility were accompanied by equally marked increases in the per cent of abnormal spermatozoa (table 5). These concurrent changes suggest that both bulls may have been ap-

TABLE 3

Average body weights and estimated daily intakes of carotene

30-day period	Body weight in lb.						Mg. carotene fed per day					
	Bulls						Bulls					
	N	*J*	*K*	*C*	*M*	*I*	*N*	*J*	*K*	*C*	*M*	*I*
Aug. '45	Ration: Poor quality roughage, hay or straw, plus low-carotene concentrate mixture											
1	1776	1246	1535	1551	1323	1083	78.1	78.1	78.1	78.1	61.0	52.7
2	1824	1269	1553	1609	1354	1135	78.1	78.1	78.1	78.1	61.0	52.7
3	1854	1281	1541	1628	1369	1188	78.1	78.1	78.1	78.1	61.0	52.7
4	1887	1337	1581	1682	1362	1211	78.1	78.1	78.1	78.1	61.0	52.7
5	1872	1350	1584	1663	1379	1224	78.1	78.1	78.1	78.1	61.0	52.7
6	.....[a]	.....	.....	.....	.....	.....	78.1	78.1	78.1	78.1	61.0	52.7
7	.....	.....	.....	.....	.....	.....	24.7	24.7	24.7	24.7	19.4	17.0
8	1866	1338	1603	1723	1307	1257	24.7	24.7	24.7	24.7	19.4	17.0
9	1847	1298	1536	1690	1226	1229	24.7	24.7	24.7	24.7	19.4	17.0
10	1801	1263	1495	1628	1198	1199	24.7	24.7	24.7	24.7	19.4	17.0
11	1792	1249	1422	1578	1167	1213	32.4	32.4	32.4	32.4	25.4	22.2
12	1832	1265	1423	1624	1195	1241	32.5	32.5	32.5	32.5	25.5	22.3
13	.....	.....	.....	.....	.....	.....	11.9	11.9	11.9	11.9	9.5	8.5
14	1903	1328	1522	1715	1247	1308	34.3	34.3	34.3	34.3	26.9	23.5
15	1897	1315	1555	1751	1289	1363	34.3	34.3	34.3	34	26.9	23.5
16	1924	1348	1588	1836	1346	1369	34.3	34.3	34.3	34.3	26.9	23.5
Dec. '46	Ration: Dried beet pulp, low carotene concentrate, plus carotene-in-oil supplement for bulls *N*, *J*, and *K*											
17	1822	1265	1520	1732	1252	1357	578	405	478	1.8	1.4	1.4
18	1825	1294	1527	1760	1269	1343	578	405	478	1.8	1.4	1.4
19	1875	1300	1485	1840	1268	1287	578	405	Slaugh-	1.8	1.4	Slaugh-
20	1958	1306	Slaugh-	1885	1296	Slaugh-	578	405	tered	1.8	1.4	tered
21	2024	1326	tered	1927	1253	tered	578	405	2–27–47	1.8	1.4	2–27–47
			2–27–47			2–27–47						

[a]..... no observations made.

TABLE 4

Average micrograms carotene and vitamin A per 100 ml. blood plasma

30-day period	γ carotene per 100 ml. plasma						γ vitamin A per 100 ml. plasma					
	Bulls						Bulls					
	N	*J*	*K*	*C*	*M*	*I*	*N*	*J*	*K*	*C*	*M*	*I*
	Ration: Poor quality roughage, hay or straw, plus low-carotene concentrate mixture											
Aug. '45												
1	114	58	105	43	79	82	21	15	10	12	9	12
2	50	52	95	42	69	70	24	17	14	16	12	13
3	38	57	81	42	70	82	17	14	9	14	14	12
4	39	59	91	41	71	94	24	21	13	21	17	16
5	34	51	99	41	69	82	17	15	13	16	15	12
6	.....[a]	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7	50	73	142	60	108	162	28	24	22	33	24	27
8	41	72	127	52	89	110	17	16	14	24	15	16
9	25	54	82	35	60	77	15	16	14	18	12	15
10	28	49	62	29	53	61	17	16	13	16	10	13
11	28	44	60	28	53	69	14	17	15	15	13	16
12	45	110	139	53	133	109	29	30	31	28	22	23
13	36	68	123	41	97	92	22	22	18	22	17	19
14	40	71	104	47	99	94	14	12	9	12	9	9
15	45	71	118	50	100	97	23	24	18	25	16	18
16	57	82	125	63	132	128	25	25	22	29	22	22
	Ration: Dried beet pulp, low carotene concentrate, plus carotene-in-oil supplement for bulls *N*, *J*, and *K*											
Dec. '46												
17	144	192	244	42	60	70	27	28	22	15	9	11
18	155	197	374	14	13	25	28	26	23	6	5	6
19	126	199	231[b]	14	19	20	24	23	6[b]	4	3	5
20	308	400	Slaughtered	13	18	Slaughtered	37	32	Slaughtered	3	3	Slaughtered
21	198	163	2–27–47	8	15	2–27–47	37	22	2–27–47	3	2	2–27–47

[a] ..... no observations made.

[b] Bloated and off feed resulting in a decreased carotene intake and probably, therefore, a lowered plasma carotene and vitamin A.

proaching a deficiency state. In the case of bull I, the average number of spermatozoa per milliliter of semen (table 5) decreased during these same periods.

During the last 5 months, when bulls *C*, *M* and *I* were on the beet pulp and low-carotene concentrate mixture without the carotene supplement, semen volume again was characterized by considerable variation without a definite trend accompanying the low plasma vitamin A values and the onset of clinical symptoms. At the same time, the per cent of motile spermatozoa decreased and the per cent of abnormal spermatozoa increased as these bulls developed advanced vitamin A deficiency (table 6).

The concentration of spermatozoa per milliliter of semen for the combined first and second ejaculates varied considerably up to the time the last samples were collected. Of those bulls receiving no carotene supplement, bull *I* exhibited an increase in spermatozoan concentration while bulls *C* and *M* exhibited decreases in concentration just prior to the time they were unable to mount.

The bulls receiving the carotene supplement exhibited sufficient variation in the several semen and spermatozoan characteristics that no particular improvement was attributed to the increased carotene intake.

Figure 1 shows photomicrographs of histological sections of the seminiferous tubules of the right testis of each bull. These photomicrographs are by pairs of bulls and show the contrasting conditions of the tubules accompanying the presence and absence of supplemental carotene in the basal ration. Bull *C* exhibited the most advanced degeneration of the germinal epithelium, followed by bull *I* and bull *M* in order. All exhibited a similar pattern of degeneration, namely, few spermatogonia, relatively small numbers of spermatocytes and immature spermatids. The seminiferous tubules of the bulls receiving the carotene supplement apparently were normal, with the exception of bull *K*, and showed numerous dividing spermatogonia, spermatocytes, maturing spermatids, and many spermatozoa in the lumen of the tubules.

It should be pointed out that the testes sections of bull *K* showed occasional tubules which were characterized by almost complete degeneration of the germinal epithelium and the absence of spermatids and spermatozoa in the lumen. Whether or not these represented unrepaired tubules arising from the feeding regime prior to the carotene supplementation is, in the absence of biopsy data, mere speculation.

Table 7 presents for comparison the observations on the onset of the clinical symptoms in terms of days after the change in roughage from hay and straw to beet pulp for those bulls not receiving the carotene supplement. Considerable variation occurred between bulls in the rapidity with which they developed incoordination, edema of the extremities and night blindness. This variation, undoubtedly, was a reflection of their respective

TABLE 5

Average semen volume per ejaculate and numbers of spermatozoa per ml.
(Averages represent first and second ejaculates)

30-day period	Semen volume per ejaculation in ml.						Numbers of spermatozoa per ml. × 10^9					
	Bulls						Bulls					
	N	*J*	*K*	*C*	*M*	*I*	*N*	*J*	*K*	*C*	*M*	*I*
	Ration: Poor quality roughage, hay or straw, plus low-carotene concentrate mixture											
Aug. '45												
1	4.3	5.6	3.9	4.1	6.3	3.8	1.21	1.23	0.60	1.09	1.16	0.89
2	6.4	5.5	6.5	4.3	5.9	3.3	1.04	1.17	0.92	0.96	1.08	0.82
3	4.4	4.2	5.8	4.3	6.8	3.1	1.45	1.11	0.78	1.54	1.13	1.05
4	3.7	5.8	7.2	3.9	8.3	2.6	1.48	1.20	0.77	1.39	1.06	0.90
5	4.5	5.2	6.6	3.5	6.9	2.5	0.99	0.99	0.76	0.81	1.31	0.81
6	3.5	6.1	7.0	5.5	6.2	2.2	1.11	1.28	0.59	1.18	1.15	0.81
7	4.3	4.6	6.0	4.6	6.6	2.5	0.79	1.14	0.59	0.88	1.12	0.61
8	3.6	4.7	5.2	5.9	6.3	2.7	1.12	0.76	0.69	1.13	0.86	0.64
9	3.9	4.4	4.4	4.8	5.5	2.1	1.21	0.90	0.52	0.74	1.08	0.49
10	3.5	3.8	5.5	4.2	5.2	3.0	0.90	1.17	1.10	0.98	0.83	0.48
11	3.6	5.4	.....[a]	5.4	4.3	1.6	1.04	1.12	.....[a]	1.40	1.06	0.38
12	2.9	2.7	.....[a]	4.0	7.1	3.4	0.92	0.80	.....[a]	1.08	1.28	0.50
13	2.7	6.2	4.6	4.1	5.9	3.4	0.71	1.16	0.62	0.97	0.68	0.64
14	3.3	4.8	2.9	4.9	5.8	3.0	0.80	0.84	0.52	1.07	0.83	0.64
15	2.9	5.5	7.5	4.6	5.9	2.8	1.00	0.94	0.74	0.77	0.93	0.44
16	4.2	5.4	8.0	4.5	7.7	3.0	0.79	1.12	0.56	1.03	1.04	0.71
	Ration: Dried beet pulp, low carotene concentrate, plus carotene-in-oil supplement for bulls *N*, *J*, and *K*											
Dec. '46												
17	4.0	6.6	6.0	4.5	6.2	3.6	0.89	0.73	0.86	0.70	1.05	0.56
18	5.9	6.9	7.1	5.6	5.7	3.7	1.28	0.76	0.90	0.97	1.27	0.97
19	6.8	6.8	6.5[c]	5.5	6.1	.....[b]	1.22	1.14	0.64[c]	0.79	1.22	.....[b]
20	6.0	7.9	Slaughtered	5.6	5.0	Slaughtered	1.17	0.87	Slaughtered	0.61	1.07	Slaughtered
21	7.4	6.7	2–27–47	3.9	3.1	2–27–47	1.14	0.83	2–27–47	0.21	0.73	2–27–47

[a] No semen collected.
[b] Unable to mount.
[c] Represents only one collection during the period.

TABLE 6

Average percentages of motile and of morphologically abnormal spermatozoa
(Averages represent first and second ejaculates)

30-day period	% motile spermatozoa						% morphologically abnormal spermatozoa					
	Bulls						Bulls					
	N	*J*	*K*	*C*	*M*	*I*	*N*	*J*	*K*	*C*	*M*	*I*
Aug. '45	Ration: Poor quality roughage, hay or straw, plus low-carotene concentrate mixture											
1	58	58	68	62	59	63	5	12	13	26	15	21
2	61	53	53	56	54	43	11	10	10	20	19	22
3	55	53	43	39	59	42	9	13	11	17	16	32
4	59	65	67	67	56	61	9	17	14	22	21	40
5	62	58	48	55	48	56	11	13	16	12	20	29
6	60	60	51	53	55	53	16	13	17	15	19	33
7	65	63	73	63	64	59	12	14	16	15	18	32
8	53	56	55	57	62	58	9	14	15	15	19	30
9	66	59	52	62	60	60	11	11	15	10	19	29
10	66	54	60	50	53	44	12	13	15	11	22	47
11	66	63	....[a]	19	61	33	10	10	....[a]	66	20	85
12	65	57	....[a]	38	54	34	8	12	....[a]	27	22	55
13	62	48	49	47	58	49	9	16	13	19	17	25
14	53	50	43	58	41	52	10	15	16	7	18	30
15	53	53	55	53	50	44	9	14	10	6	16	27
16	50	46	45	53	43	47	7	12	12	10	15	24
	Ration: Dried beet pulp, low carotene concentrate, plus carotene-in-oil supplement for bulls *N*, *J*, and *K*											
Dec. '46												
17	60	48	45	57	51	31	7	9	9	8	11	25
18	65	47	53	44	44	29	6	8	9	12	12	24
19	52	46	30[c]	40	44	....[b]	6	8	8[c]	20	13	....[b]
20	56	53	Slaughtered 2-27-47	36	36	Slaughtered 2-27-47	4	9	Slaughtered 2-27-47	31	26	Slaughtered 2-27-47
21	67	45		45	20		3	12		64	68	

[a] No semen collected.
[b] Unable to mount.
[c] Represents only one collection during the period.

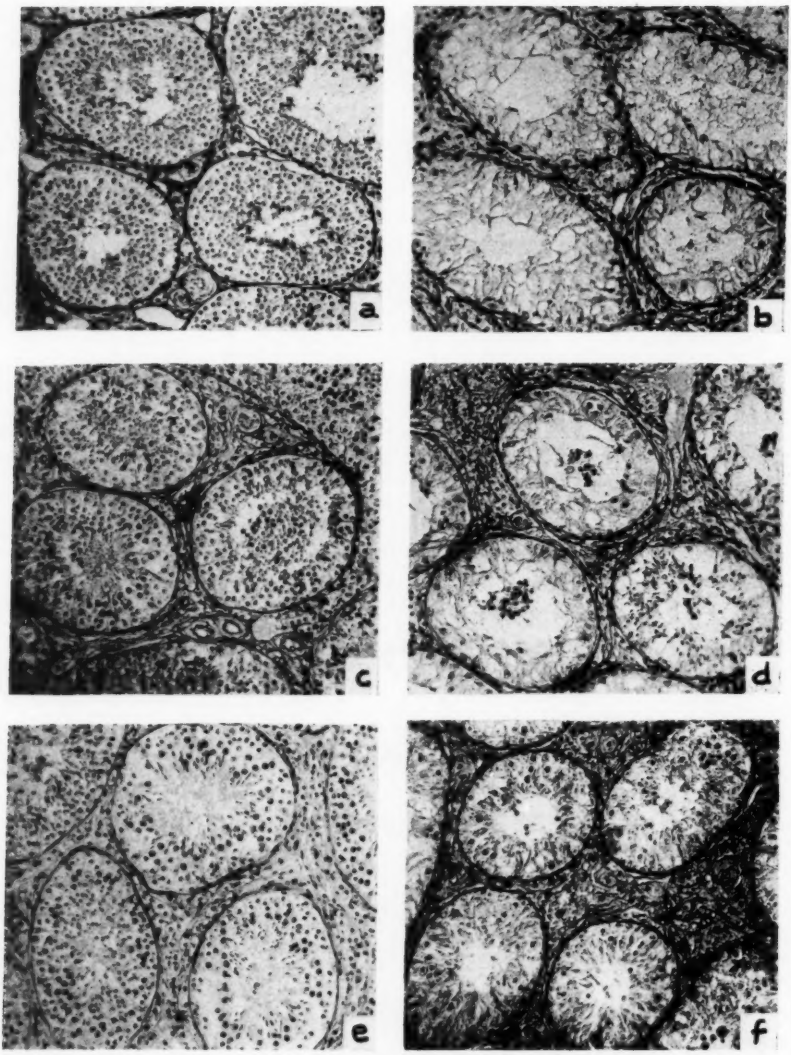

FIG. 1. Photomicrographs of seminiferous tubules of the right testis of each bull at time of slaughter. (×100.) a, c and e—bulls *N*, *K* and *J* after 151, 73 and 151 days, respectively, on the ration supplemented with carotene. b, d and f—bulls *C*, *I* and *M* after 151, 73 and 151 days, respectively, on the ration unsupplemented with carotene.

TABLE 7

Number of days bulls were fed deficient ration before onset of first clinical symptoms of vitamin A deficiency

Bull	Off feed	First signs of incoordination	Unable to mount	Extreme incoordination	Stiffness in legs	Marked edema of knees hocks, and ankles	Night blindness[a]
C	...[b]	90	140	140	130–140	150	150[c]
M	38[d]	120	150[e]	150	150	.....[f]	150[g]
I	38	40	50–60	70	60	70	.....[f]

[a] All bulls on the unsupplemented ration showed varying degrees of characteristic bleaching of the tapetum lucidum.
[b] Bull *C* was never off feed.
[c] Hemorrhagic areas about the papilla of right eye.
[d] Bull *M* was off and on feed several times during the course of the feeding of the deficient diet. Scouring was also a frequent accompaniment of his erratic appetite.
[e] Bull *M* was sufficiently incoordinated that he undoubtedly would not have been able to mount in another 10 days.
[f] Condition not in evidence at completion of experiment.
[g] It was questionable whether this bull was actually night blind.

body stores of carotene and vitamin A and their rates of depletion on the experimental rations employed.

In general, it would appear that incoordination and loss of the ability to mount, without the loss of libido, are the earliest signs of vitamin A deficiency in mature breeding bulls and occur before any marked impairment in semen production is manifested.

The liver stores of carotene and vitamin A at the time of slaughter are shown in table 8. The values reflect clearly the rations fed and the breeds used.

From the evidence of this investigation and the reports of others, the

TABLE 8

Carotene and vitamin A content of the fresh livers at the time of completion of experiment for individual bulls

Bull	Breed	No. of days between date of change of ration and date of slaughter	γ per g. fresh liver	
			Carotene	Vitamin A
	Ration: Dried beet pulp, low carotene concentrate mixture, plus daily supplement of carotene-in-oil			
N	Holstien	151	438	778
J	Guernsey	151	1335	273
K	Guernsey	73	873	73
	Ration: Dried beet pulp and low-carotene concentrate mixture			
C	Brown Swiss	151	28	12
M	Guernsey	151	48	13
I	Guernsey	73	47	5

authors believe that, while typical clinical manifestations of vitamin A deficiency and degeneration of the germinal epithelium can be produced in mature dairy bulls, the feeding of poor quality dry roughages, presumably low in carotene, for extended periods of time will not likely cause noticeable impairment of semen production before the onset of the clinical manifestations of A deficiency.

SUMMARY AND CONCLUSIONS

Six dairy bulls were fed dry roughages low in carotene and a concentrate deficient in carotene and vitamin A for a period of 16 months without inducing clinical manifestations of vitamin A deficiency or noticeable impairment of semen production.

Subsequent changes in the roughage component of the ration of three of the bulls from hay and/or straw to dried beet pulp plus the same concentrate mixture resulted in the development of incoordination, edema of the extremities, papillary hemorrhage, a gradual increase in the per cent of abnormal spermatozoa and a decrease in the per cent of motile spermatozoa, but no consistent change in the number of spermatozoa per milliliter of semen within a period of 40 to 120 days. These three bulls were unable to mount but still retained an unusual amount of libido. This inability to mount was manifested before the changes in semen production.

Typical patterns of degeneration of the germinal epithelium of the seminiferous tubules were found in the three bulls on the carotene-deficient rations. There were few spermatogonia, spermatocytes, spermatids or maturing spermatozoa in the lumen of the tubules.

While supplementing the carotene-deficient ration of the other three bulls with carotene in oil prevented the degeneration of the germinal epithelium which characterized those bulls not receiving the supplement of carotene in oil, it produced no consistent changes in semen production which reasonably could be attributed to the carotene.

Liver carotene and vitamin A levels of the carotene-deficient bulls were of the order of 30 to 50 γ and 5 to 13 γ per g. of fresh liver, respectively.

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FURTHER STUDIES OF THE NUTRITIVE VALUE OF BUTTERFAT FRACTIONS[1]

H. NATH,[2] V. H. BARKI, C. A. ELVEHJEM, AND E. B. HART
Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

It was reported (4) that butterfat collected in September, 1945, was fractionated by crystallization from acetone at −4° C. and yielded two fractions; a liquid one (II) which promoted growth in rats at a superior rate as compared with butterfat or corn oil, and a solid one (I) which allowed a significantly inferior growth rate. This phenomenon was not observed to the same degree in later trials when samples of butter collected in subsequent months, including September, 1946, were used. Since the pasture of the summer of 1947 was considered as generally better than that of 1946, it was thought advisable to repeat the same fractionation procedure using the late summer butterfat from cows on this better pasture.

Fractionation of milk fat by cold crystallization also has been reported by Jack *et al.* In their early work (5) they did not obtain any one fraction with a growth promoting action superior to that of the whole milk fat. In a recent paper (6), they report that by the use of purified solvents they obtained a milk fat fraction which produced a significantly better growth in young rats than the original milk fat and also greater growth than supported by the other milk fat fractions. They attributed their first results to the effect of impurities in the solvents on the fat.

EXPERIMENTAL

The September, 1947, butter obtained from the University dairy was fractionated in the same manner described in the first paper of this series (1), again using purified acetone as the solvent, and at a temperature of −5° C. Fraction I had a melting point of 42° C., and an iodine number of 21, fraction II, a melting point of 7° C., and an iodine number of 51.

The fractions, as well as the untreated butterfat and corn oil, were used as the separate sources of fat in the diets fed to four groups of six rats each. Male weanling rats of the Sprague-Dawley strain weighing 40–50 g. were placed on the diets, the basic composition of which is shown in table 1.

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[2] Government of India Research Fellow.

TABLE 1

Composition of the diets

Components	28% Diet	10% Diet
Fat[a]	28	10
Sucrose	48	66
Casein[b]	20	20
Salts IV[c]	4	4
Vitamin supplement mg. per 100 g. diet		
Thiamine		0.2
Riboflavin		0.3
Pyridoxine		0.3
Ca pantothenate		1.5
Choline hydrochloride		150
β-Carotene[d]		0.56
α-Tocopherol		2.24
Calciferol[e]		.....
2-Methol-1,4-naphthoquinone		0.21

[a] Butterfat or corn oil or one of the fractions.
[b] Extracted for three 2-hour periods with boiling 95% alcohol.
[c] Phillips, P. H., and Hart, E. B., J. Biol. Chem., 109: 657. 1936.
[d] 90% β-carotene and 10% α-carotene.
[e] Crystalline irradiated ergosterol.

The fat level of the diet in this experiment was 28 per cent. The rats were housed in individual metal cages with raised screen bottoms, watered and fed daily *ad libitum* and weighed once weekly. Consumption records were kept during the fifth week, but since the growth of the groups on the corn oil and fraction I diets was irregular during this week, only the efficiency of the butterfat and fraction II diets was calculated.

Table 2 gives the gain in weight of the rats for 5- and 6-week periods. It also includes the efficiency of each of the two diets mentioned above in terms of grams of diet consumed per gram of weight gained.

Even though the differences in growth in this experiment were not as wide as those obtained in 1945, fraction II again showed a striking superiority over fraction I, and over the butterfat and corn oil. Furthermore, the efficiency of the diet containing this fraction (II) was found higher than that containing whole butterfat. On comparing the gains on the butterfat itself to those on the corn oil, some superiority of the former is apparent with these sucrose diets.

TABLE 2

Growth gains and efficiency values on 28 per cent fat diets

	Butterfat	Corn Oil	Fraction I	Fraction II
	g.	*g.*	*g.*	*g.*
5-week gain	170	165	149	186
6-week gain	190	184	168	217
Grams diet/gram gain during 5th week	2.5	.....	.....	2.22

In repeating this experiment for further verification, it was decided to replace the corn oil diet by another containing Crisco (a partially hydrogenated cottonseed oil) as the source of fat. This was done in view of the reports by Kentie (7) and Boer *et al.* (2), (3), who claimed that the superiority of summer butter is due to its content of vaccenic acid and also because it was possible by Bertram's method (1) to isolate from Crisco a substance presumably corresponding to vaccenic acid. Also, the entire series was repeated on diets containing 10 per cent fat (table 1) in order to investigate the possibility that while a growth-promoting factor is being concentrated in fraction II, a retarding growth factor might be left in fraction I, and when fed at a 28 per cent level, would inhibit growth. Table 3 represents the various diets used and the corresponding weight gains during a 5-week period. During the sixth week, some of the rats were killed for microbiological analysis of their cecal flora.

TABLE 3

Growth gains during the 5-week period on 28 and 10 per cent fat diets

	Butterfat	Crisco	Fraction I	Fraction II
28% fat	143	144	.....	157
10% fat	146	137	130	161

In comparing the values of the first experiment in table 2 to those displayed in table 3, generally poorer growth of the latter series becomes obvious. This is an unfortunate phenomenon in this type of study, and one which emphasizes the need for large numbers of experimental animals. However, within this series, the superiority of fraction II over the butterfat itself again is indicated both on the 28 and the 10 per cent level. The Crisco, from which have been isolated large amounts of the substance presumably corresponding to vaccenic acid, did not stimulate better growth than did butterfat.

In this experiment no significant difference seemed to have resulted from the varying levels of fat in the diets. However, work in progress points to the probability of the presence in certain fats of an inhibiting factor which exerts its effect to a noticeable degree when the fat represents a higher portion of the diet.

SUMMARY

September butterfat (1947) again was fractionated by crystallization from acetone at $-5°$ C., yielding a liquid fraction (II) which allowed rats to grow at a superior rate as compared with butterfat or corn oil, and a solid fraction (I) which gave a significantly slower growth rate.

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SACRAL DEFORMITY IN THE "WRYTAIL" ABNORMALITY IN CATTLE[1]

LESTER O. GILMORE AND ALVIN F. SELLERS[2]
Divisions of Dairy Husbandry and Veterinary Medicine,
Minnesota Agricultural Experiment Station,
St. Paul, Minnesota

The importance and the possible mode of inheritance of "wrytail" in cattle have been presented by Atkeson and Warren (2) and by Atkeson *et al.* (1). These workers point out that the "wrytail" character has been observed in the Guernsey, Holstein-Friesian, Ayrshire, Brown Swiss and Jersey breeds. Their data indicate its inheritance as a single autosomal recessive. Observations of the present authors have extended to four breeds heretofore unreported and include 137 cattle of different ages and both sexes, as follows: Beef Shorthorns, 51; Aberdeen Angus, 39; Hereford, 17; Red Polled, 30. The only case of wrytail found was in one Red Polled cow in which the tailhead was set to the left.

A "wrytail", according to Atkeson *et al.* (1) is a malformation consisting of a distortion of the tail head, the base of the tail being set at an angle to the back bone instead of in line with it. The "tailhead" is the term commonly used by dairy cattle workers and judges to designate the area limited by the first three coccygeal vertebrae. The "wrytail" malformation therefore, would seem to be at or near the junction of the fifth sacral and first coccygeal vertebrae.

Roemmele (5) described a condition in Brown Allgauer cattle as being somewhat similar to "wrytail". Essentially, this malformation involved a twisting of the coccygeal vertebrae at a point posterior to the tail head. While the malformation described by Roemmele is similar in the effect on the vertebrae and intervertebral discs, the region affected makes it more nearly resemble "screwtail" (Knapp *et al.* (4)) than "wrytail".

EXPERIMENTAL

The present investigation involves a study of the anatomical features of the "wrytail" condition in a 7-year-old purebred Jersey female. Inasmuch as this was a case of definite wrytail and no similar analysis is known to have been attempted, it is thought the results may be of interest to others. In this case the tail was set to the right as indicated in figure 1. This condition was first observed when she was a 2-year-old. It is not

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known how long the character was present or if it was present at birth, as prior observations with this in mind had not been made. Three months after this picture was taken, the cow was slaughtered and a section of the rump, approximating 60 lb., was removed. This portion included the sacrum, most of the tail, and parts of the ilia. From the tissues thus involved, that portion of the tail and sacrum shown in figure 2 was prepared by removing the soft tissue, first by boiling water and, subsequently, by removing soft tissue with a scalpel and, finally, with steel wool. The sacrum was

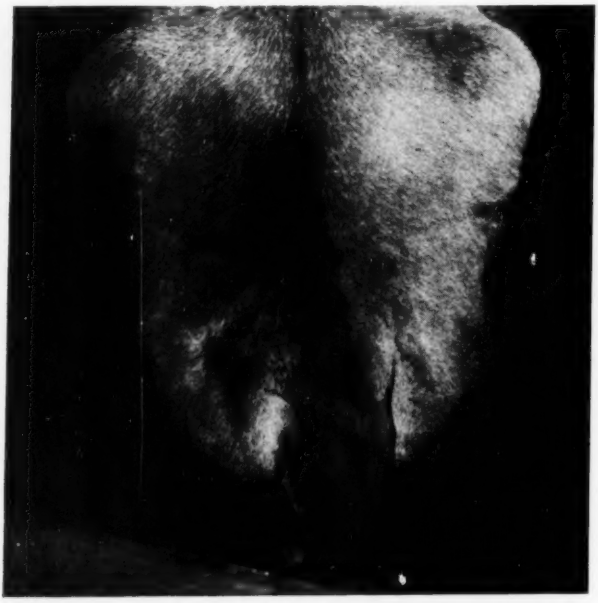

FIG. 1. Dorsal aspect of sacro-coccygeal region on a 7-year-old multiparous Jersey cow, no. 264.

then compared with the normal as described by Ellenberger and Baum (3) and by Sisson and Grossman (6). No evidence of trauma or inflammatory change (in terms of callus formation, thickening, or exostosis) was discovered.

The sacrum in this subject was 25 cm. in over-all length, 19 cm. in width anteriorly (alae) and 7.8 cm. in width at the extreme posterior extremity. Sacral segments I–IV, inclusive, were fused in their bodies, and in their spinous, transverse, and articular processes. The over-all length represented by fused segments I–IV, inclusive, was 20 cm. The fifth sacral segment was not fused at the junction of its body or transverse,

spinous, or articular processes with those of the fourth segment. The fifth dorsal and ventral sacral foramina thus were incomplete and that part of the lateral sacral crest, contributed to by the articular processes of sacral segments IV and V, also was incomplete. A lateral declination of approximately 12° to the right from the longitudinal axis common to sacral segments I–III, inclusive, to the axis common to sacral segments IV and V was noted. No declination was noted between the longitudinal axis of

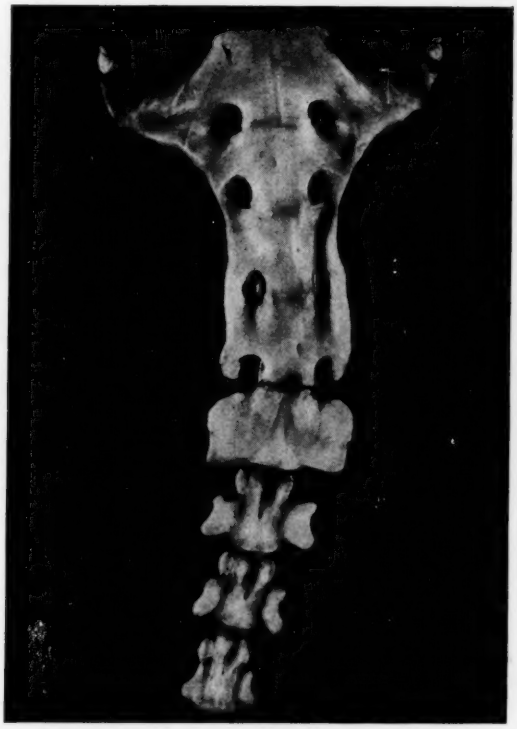

FIG. 2. Ventral aspect of sacrum and first three coccygeal vertebrae of Jersey cow no. 264, showing sacral deformity.

sacral segment V and that possessed in common by the first three coccygeal vertebrae (fig. 3).

Three radiographs were taken of this area, one before death and two after death. The one taken during life (11–15–46) demonstrated that the deformity did not lie in the anterior coccygeal region. The second (fig 3), taken of the frozen rump, revealed the site of the deformity to be in the sacrum. The final radiograph (fig. 4) was made of the sacro-coccygeal re-

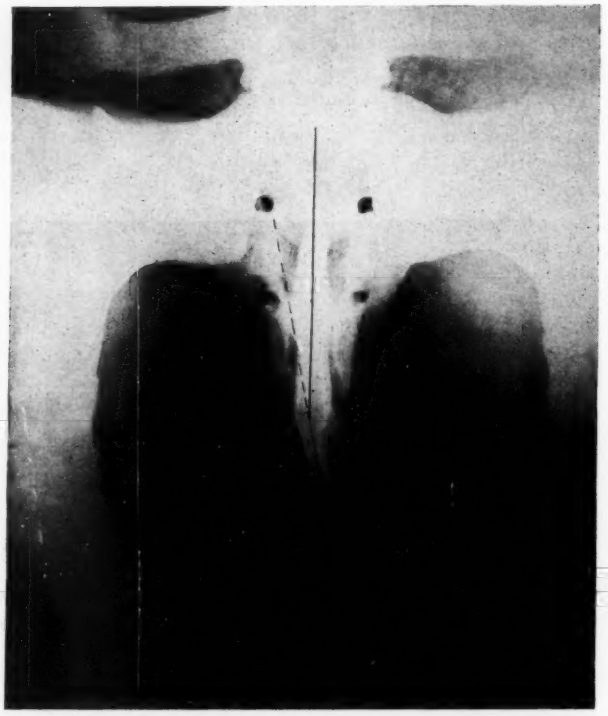

FIG. 3. Radiograph of sacro-coccygeal region, in section of rump removed from carcass at slaughter and then frozen. Dorsal view. Cow no. 264.

gion after removing the soft tissue. The specimen was laid flat on the cassette (*i.e.*, with the sacral alae depending over the edge) and a series of exposure made of the area apparently involved in the deformity (segments III–V, inclusive). The exposures were made on six portions of one large

TABLE 1

Detailed radiographic study of sacral segments III–V, inclusive

Exposure no.	Voltage	Distance	Tube current reading	Time
	(*kilovolts*)	(*in.*)	(*milliamps.*)	(*sec.*)
1	60	30	52	0.1
2	60	30	52	0.25
3	60	30	52	0.5
4	40	30	55	0.25
5	40	30	55	0.5
6	40	30	55	0.75

film, using lead plates to delineate the areas. Time and intensity variations were introduced, as noted in table 1.

The first series (exposures 1–3, inclusive) showed greater delineation of articulation or fusion (as the case might be) of adjacent segments, and midline detail in general. The second series (exposures 4–6, inclusive) showed greater delineation of transverse process structure.

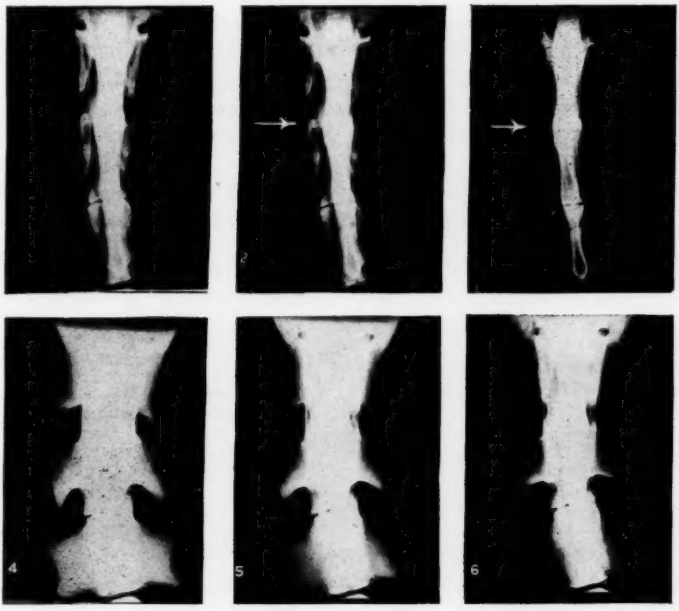

FIG. 4. Detailed radiographic study of sacral segments III–IV, Dorsal view (see table 1). Arrow indicates wedge-shaped area of fusion between segments III–IV. Cow no. 264.

An over-all lateral declination (right) of about 12° was noted, occurring at the fusion point of segments III–IV (7°) and within segment IV itself (5°). Since the specimen lay flat on the casette at the time of exposure, and since the anode-film distance was relatively great and the perpendicularity of the central ray to the film was carefully checked, it was felt that linear and angular measurements made from the film would be valid. A measurement of declination of involved segments, made from the film (exposure 3, fig. 4), showed approximately 7° lateral declination (right) of the longitudinal axis (perpendicular to the line of fusion) of the anterior portion of sacral segment IV, from the longitudinal axis of segment III. An additional lateral declination of approximately 5°

(right) of the longitudinal axis of the posterior portion of segment IV (also perpendicular to the transverse axis of the articulation concerned, *i.e.*, IV–V) from the longitudinal axis of the anterior portion, was noted.

On exposure 3, figure 4, a measurement of the intersegmental distance at the fusion point demonstrated a slight wedge-shape of that area. At its central and right portions, this area measured approximately 2 mm. in antero-posterior thickness. At its visibly expanded left extremity, it measured 3 mm. in antero-posterior thickness. The right lateral portion was not as amenable to accurate measurement on the X-ray film as the other portions of the fusion area, due to the partial obscurity cast by the spinous process. However, the wedge-shape and general measurements of this area were verified on the specimen itself. External measurements on the specimen are given in table 2.

Measurements also were made on right and left sagittal longitudinal axes of segment IV, at a distance of 12 mm. to either side of the ventral

TABLE 2

External measurements of intersegmental fusion areas of sacrum

Articulation (fusion area) of sacral segments	Transverse diameter of articulation[a]	Ventral intersegmental distance, antero-posterior		
		Left, 12 mm. from midpoint	Mid-point	Right, 12 mm. from midpoint
	(*mm.*)	(*mm.*)	(*mm.*)	(*mm.*)
I–II	39	5.0	2.0	5.0
II–III	35	3.5	2.75	3.25
III–IV	28	3.0	2.5	2.0

[a] Calipers and dividers were used in making measurements.

midline (the distance 12 mm. was chosen arbitrarily, since differences seemed fairly pronounced and more easily measurable at that distance). The length of the right sagittal axis was found to be 47 mm. as compared to 50 mm. for the length of the corresponding left sagittal axis.

CONCLUSIONS

Examination of the sacral and coccygeal vertebrae of one animal showed the "wrytail" malformation to involve the sacrum rather than the tailhead in the case studied.

The extent and direction of the malformation as measured by the angle of declination was 12° right.

From radiographic and other observations, it appears that one locus of bone growth disparity lay in the fusion area between sacral segments III and IV. An additional declination (42 per cent of the total) occurred within the body of sacral segment IV.

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FROZEN HOMOGENIZED MILK. IV. KEEPING QUALITY OF FROZEN HOMOGENIZED MILK AFTER THAWING

C. J. BABCOCK,[1] CAPT. JOSEPH N. STABILE, SnC, USA, EARNEST WINDHAM, LARUE B. EVANS, AND COL. RAYMOND RANDALL, VC, USA

Veterinary Division, Army Medical Department Research and Graduate School, Army Medical Center, Washington, D. C.

Previous studies (2, 5, 6, 7, 9) have shown that freezing and storage temperatures affect the physical character of homogenized milk. Later studies (3, 4, 8) showed that when homogenized milk was frozen, the solid components tended to concentrate in the lower portion of the sample. However, changes in the temperature at which frozen homogenized milk was stored did not affect materially the chemical composition of the different sections of quart samples. The concentration of the milk solids in the lower portion of the sample took place during the freezing process and apparently there was no further movement of these solids after the milk was frozen.

The literature does not contain information regarding the keeping quality of homogenized milk after it has been frozen and then thawed. The present study therefore was undertaken to determine the effect that storage of frozen homogenized milk has on its keeping quality after thawing.

PROCEDURE

Homogenized milk samples with a fat content of 3.8 per cent packaged in one-half pint paper containers by a commercial dairy in Washington, D. C., were used. This milk had been pasteurized at 155° F. for 30 minutes.

Samples of the fresh homogenized milk immediately were stored at 30.5° C., 15.5° C., 12.8° C., 7.22° C., and 1.67° C. Other samples were frozen immediately and held at – 27.5° C. for various periods, after which they were thawed and then stored at the same temperatures as those at which the fresh milk samples had been held. At regular intervals a one-half pint sample of milk was removed from storage in order to determine its bacterial content, titratable acidity, pH, and flavor. The first three of these determinations were made in accordance with the methods outlined in Standard Methods for the Examination of Dairy Products (1). The plates were incubated at 37° C. for 48 hours. Flavor determinations were made by a panel of three men experienced in milk judging.

RESULTS

The flavor developments in homogenized milk held for different periods of time before and after freezing are shown in table 1. This table shows

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[1] Formerly Lieutenant Colonel, SnC, AUS. Present address: Dairy Branch, Production and Marketing Administration, U. S. Department of Agriculture.

TABLE 1

Flavor of homogenized milk before and after freezing

Storage temperature	Storage period	Flavor quality										
		Before freezing	No. of days frozen milk stored at −27.5° C.									
			3	17	24	31	45	66	87	111	129	150
(° *C.*)	(*days*)											
Fresh	.....	Normal	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
30.5	1	Sour	.....	.....	.....	.....	.....	Sour	Sour	Sour–Unclean	Sour–Putrid	.....
15.5	1	Normal	Normal	.....	.....	.....	.....	.....	.....	.....	.....	.....
	2	Normal	Normal	.....	.....	.....	.....	.....	.....	.....	.....	.....
	3	Normal	Normal	.....	.....	.....	.....	.....	.....	.....	.....	.....
	4	High acid.	Sl. oxidized	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fresh	.....	Normal	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12.8	1	Normal	Normal	.....	.....	.....	.....	.....	.....	.....	.....	.....
	2	Normal	Normal	.....	.....	.....	.....	.....	.....	.....	.....	.....
	4	Normal	Normal	.....	.....	.....	.....	.....	.....	.....	.....	.....
	5	Normal	Normal	.....	.....	.....	.....	.....	.....	.....	.....	.....
	6	Normal	Sl. old	.....	.....	.....	.....	.....	.....	.....	.....	.....
	7	Sl. old	Sl. old	.....	.....	.....	.....	.....	.....	.....	.....	.....
	8	Sl. old	Old	.....	.....	.....	.....	.....	.....	.....	.....	.....
7.22	2	Normal	Normal	Normal	Normal	.....	.....	.....	.....	.....	.....	.....
	4	Normal	Normal	Normal	Normal	Normal	Normal	.....	.....	.....	.....	.....
	6	Normal	Normal	Normal	Sl. old	Normal	Normal	.....	.....	.....	.....	.....
	8	Sl. old	Normal	Sl. old	Sl. old	Normal	Sl. old	.....	.....	.....	.....	.....
	10	Sour	Old	.....	.....	.....	.....	.....	.....	.....	.....	.....
	12	Sour	Sour	High acid	Sour	Old	Sour	.....	.....	.....	.....	.....
1.67	2	Normal	.....	.....	.....	.....	.....	Normal	Normal	.....	Normal	Normal
	4	Normal	.....	.....	.....	.....	.....	Normal	Normal	Normal	Normal	Normal
	7	Normal	.....	.....	.....	.....	.....	Normal	Normal	Normal	Normal	Normal
	9	Normal	.....	.....	.....	.....	.....	Normal	Normal	Normal	Sl. old	Sl. old
	11	Sl. old	.....	.....	.....	.....	.....	.....	Normal	Sl. old	.....	.....
	14	Sl. old	.....	.....	.....	.....	.....	Sl. old	Sl. old	Old	Sl. old	Sl. old
	16	Old	.....	.....	.....	.....	.....	Sl. old	Sl. old	.....	Old	.....
	18	Old–Unclean	.....	.....	.....	.....	.....	Old	Old	.....	.....	.....
	21	.....	.....	.....	.....	.....	.....	Old–Bitter	Old–Unclean	.....	.....	.....

TABLE 2

Bacterial growth in homogenized milk before and after freezing

Storage temperature	Storage period	Bacteria per millimeter										
		Before freezing	No. of days frozen milk stored at −27.5° C.									
			3	17	24	31	45	66	87	111	129	150
(°C.)	(*days*)											
Fresh	.....	800	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
30.5	1	11,600,000	.....	.....	.....	.....	.....	11,248,000	31,400,000	70,000,000	30,000,000	.....
15.5	1	850	600	.....	.....	.....	.....	.....	.....	.....	.....	.....
	2	6,500	1,300	.....	.....	.....	.....	.....	.....	.....	.....	.....
	3	278,000	133,000	.....	.....	.....	.....	.....	.....	.....	.....	.....
	4	4,000,000	1,980,000	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fresh	.....	300	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12.8	1	400	400	.....	.....	.....	.....	.....	.....	.....	.....	.....
	2	400	500	.....	.....	.....	.....	.....	.....	.....	.....	.....
	4	800	300	.....	.....	.....	.....	.....	.....	.....	.....	.....
	5	5,300	300	.....	.....	.....	.....	.....	.....	.....	.....	.....
	6	.....	4,800	.....	.....	.....	.....	.....	.....	.....	.....	.....
	7	975,000	80,000	.....	.....	.....	.....	.....	.....	.....	.....	.....
	8	560,000	351,000	.....	.....	.....	.....	.....	.....	.....	.....	.....
7.22	2	490	1,300	400	900	.....	.....	.....	.....	.....	.....	.....
	4	7,000	600	600	300	400	300	.....	.....	.....	.....	.....
	6	152,000	123,000	.....	117,000	167,000	90,000	.....	.....	.....	.....	.....
	8	2,770,000	160,000	69,000	780,000	140,000	134,000	.....	.....	.....	.....	.....
	10	6,000,000	220,000	4,860,000	11,000,000	165,000	500,000	.....	.....	.....	.....	.....
	12	12,000,000	3,500,000	21,500,000	92,000,000	310,000	1,230,000	.....	.....	.....	.....	.....
1.67	2	800	.....	.....	.....	.....	.....	300	1,000	.....	1,600	.....
	4	1,000	.....	.....	.....	.....	.....	300	200	300	1,000	1,100
	7	1,200	.....	.....	.....	.....	.....	300	500	500	600	1,900
	9	1,600	.....	.....	.....	.....	.....	300	400	1,100	600	7,400
	11	5,000	.....	.....	.....	.....	.....	11,200	12,000	123,000	.....	36,000
	14	180,000	.....	.....	.....	.....	.....	78,000	11,400	95,800	600,000	13,800,000
	16	.....	.....	.....	.....	.....	.....	160,000	.....	.....	1,000,000	.....
	18	960,000	.....	.....	.....	.....	.....	420,000	342,000	.....	.....	.....
	21	1,500,000	.....	.....	.....	.....	.....	1,000,000	780,000	.....	.....	.....

that storage has practically the same effect on the flavor of both the fresh homogenized milk and the homogenized milk that had been held in the frozen state. The samples that were stored at 30.5° C. were sour when examined at the end of 24 hours. Those which previously had been stored for 111 days and 129 days at −27.5° C. were unclean or putrid, indicating that the rate of flavor deterioration may be slightly more rapid in milk that has

TABLE 3

Titratable acidity development in homogenized milk before and after freezing
(Standard methods (1) procedure used)

Storage temperature	Storage period	Titratable acidity (% lactic acid)									
		Before freezing	No. of days frozen milk stored at −27.5° C.								
			3	17	24	31	45	66	87	111	129	150
(°C.)	(*days*)											
Fresh	.....	0.12	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
30.5	1	0.36	.....	.....	.....	.....	.....	0.37	0.36	0.34	0.35	.....
15.5	1	0.12	0.12	.....	.....	.....	.....	.....	.....	.....	.....	.....
	2	0.12	0.12	.....	.....	.....	.....	.....	.....	.....	.....	.....
	3	0.12	0.12	.....	.....	.....	.....	.....	.....	.....	.....	.....
	4	0.17	0.16	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fresh	.....	0.12	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12.8	1	0.12	0.12	.....	.....	.....	.....	.....	.....	.....	.....	.....
	2	0.12	0.12	.....	.....	.....	.....	.....	.....	.....	.....	.....
	4	0.12	0.13	.....	.....	.....	.....	.....	.....	.....	.....	.....
	5	0.12	0.13	.....	.....	.....	.....	.....	.....	.....	.....	.....
	6	.....	0.13	.....	.....	.....	.....	.....	.....	.....	.....	.....
	7	0.13	0.13	.....	.....	.....	.....	.....	.....	.....	.....	.....
	8	0.14	0.12	.....	.....	.....	.....	.....	.....	.....	.....	.....
7.22	2	0.12	0.12	0.12	0.12	.....	.....	.....	.....	.....	.....	.....
	4	0.12	0.12	0.12	0.12	0.12	0.12	.....	.....	.....	.....	.....
	6	0.13	0.13	.....	0.13	0.12	0.13	.....	.....	.....	.....	.....
	8	0.14	0.13	0.13	0.14	0.13	0.15	.....	.....	.....	.....	.....
	10	0.28	0.13	0.17	0.16	0.15	0.16	.....	.....	.....	.....	.....
	12	0.45	0.27	0.20	0.33	0.16	.....	.....	.....	.....	.....	.....
1.67	2	0.12	.....	.....	.....	.....	.....	0.13	0.13	.....	0.13	0.14
	4	0.12	.....	.....	.....	.....	.....	0.12	0.13	0.12	0.15	0.14
	7	0.13	.....	.....	.....	.....	.....	.....	0.12	0.12	.....	0.14
	9	0.13	.....	.....	.....	.....	.....	0.14	0.12	0.12	0.15	0.14
	11	0.14	.....	.....	.....	.....	.....	0.13	0.12	0.13	0.15	.....
	14	0.15	.....	.....	.....	.....	.....	0.13	0.12	0.17	0.14	0.14
	16	.....	.....	.....	.....	.....	.....	0.15	0.13	.....	.....	.....
	18	0.18	.....	.....	.....	.....	.....	0.18	0.15	.....	.....	.....
	21	0.20	.....	.....	.....	.....	.....	.....	0.16	.....	.....	.....

been held in the frozen state. However, there appeared to be no appreciable difference in the rate of flavor deterioration between the two milks when lower storage temperatures were used.

Table 1 further shows that frozen homogenized milk of good quality can be stored at usual storage temperatures after thawing without deterioration in flavor for longer periods of time than usually are required to hold fluid milk before use.

To determine whether bacteria multiply faster and acidity develops more rapidly in homogenized milk which has been frozen and then thawed than in the corresponding fresh homogenized milk, samples of the two milks were stored at different temperatures. The initial bacterial count, acidity, and pH values were determined prior to placing the milks in storage. After different storage periods, these determinations were repeated. The results are given in tables 2, 3 and 4.

TABLE 4

pH changes in homogenized milk before and after freezing

Storage temperature	Storage period	pH									
		Before freezing	No. of days frozen milk stored at −27.5° C.								
			3	17	24	31	45	66	87	111	129	150
(°C.)	(*days*)											
Fresh	.....	6.73	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
30.5	1	5.40	.....	.....	.....	.....	.....	5.28	5.43	5.96	5.46	.....
15.5	1	6.65	6.52	.....	.....	.....	.....	.....	.....	.....	.....	.....
	2	6.61	6.52	.....	.....	.....	.....	.....	.....	.....	.....	.....
	3	6.52	6.57	.....	.....	.....	.....	.....	.....	.....	.....	.....
	4	6.37	6.43	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fresh	.....	6.70	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12.8	1	6.69	6.18	.....	.....	.....	.....	.....	.....	.....	.....	.....
	2	6.61	6.65	.....	.....	.....	.....	.....	.....	.....	.....	.....
	4	6.33	6.51	.....	.....	.....	.....	.....	.....	.....	.....	.....
	5	6.70	6.43	.....	.....	.....	.....	.....	.....	.....	.....	.....
	6	.....	6.46	.....	.....	.....	.....	.....	.....	.....	.....	.....
	7	6.60	6.45	.....	.....	.....	.....	.....	.....	.....	.....	.....
	8	6.44	6.47	.....	.....	.....	.....	.....	.....	.....	.....	.....
7.22	2	6.63	6.52	6.55	6.46	.....	.....	.....	.....	.....	.....	.....
	4	6.52	6.57	6.50	6.37	6.64	6.50	.....	.....	.....	.....	.....
	6	6.58	6.55	.....	6.42	6.43	6.42	.....	.....	.....	.....	.....
	8	6.50	6.46	6.50	6.52	6.52	6.48	.....	.....	.....	.....	.....
	10	6.01	6.52	6.23	6.48	6.48	6.48	.....	.....	.....	.....	.....
	12	5.34	5.84	6.00	5.82	6.48	.....	.....	.....	.....	.....	.....
1.67	2	6.50	.....	.....	.....	.....	.....	6.57	6.61	.....	6.62	6.74
	4	6.58	.....	.....	.....	.....	.....	6.58	6.73	6.64	6.80	6.68
	7	6.66	.....	.....	.....	.....	.....	.....	6.62	6.62	.....	6.48
	9	6.50	.....	.....	.....	.....	.....	6.58	6.68	6.52	6.68	6.50
	11	6.48	.....	.....	.....	.....	.....	6.56	6.70	6.60	6.62	.....
	14	6.50	.....	.....	.....	.....	.....	6.58	6.72	6.54	6.62	6.69
	16	.....	.....	.....	.....	.....	.....	6.52	6.62	.....	.....	.....
	18	6.40	.....	.....	.....	.....	.....	6.49	6.52	.....	.....	.....
	21	5.90	.....	.....	.....	.....	.....	.....	6.48	.....	.....	.....

Table 2 shows that, as previously reported (3), freezing milk and storing it in the frozen state had a tendency to lower the number of bacteria per ml. as determined by the standard plate count. The initial bacterial counts of the fresh homogenized milk were higher than those of the corresponding homogenized milk which had been held in the frozen state. These differences, however, were not consistently reflected in the counts made after various periods of storage. This indicates that from a bacteriological standpoint there is no significant difference between fresh homogenized milk and homogenized milk which has been held in the frozen state.

Tables 3 and 4 show that acidity develops at practically the same rate in homogenized milk when thawed, after being held in the frozen state, as it does in fresh homogenized milk when both are held under similar conditions.

CONCLUSIONS

The changes in flavor during the storage of homogenized milk which has been held in the frozen state and then thawed were similar to those of fresh homogenized milk. Frozen homogenized milk of good quality may be stored at usual storage temperatures after thawing without deterioration in flavor for longer periods than usually are required to hold fluid milk before use.

From a bacteriological standpoint, as determined by standard plate counts, there was no significant difference between homogenized milk which has been stored in the frozen state and then thawed and fresh homogenized milk.

Homogenized milk that had been stored in the frozen state and then thawed showed no significant difference from fresh homogenized milk in the development of acid as measured by titratable acidity and pH determinations.

The authors wish to express their appreciation to Edith Giltner and Elmina Dickson for their assistance with the analytical determinations.

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FROZEN HOMOGENIZED MILK. V. EFFECT OF AGE BEFORE FREEZING ON THE KEEPING QUALITY OF FROZEN HOMOGENIZED MILK

C. J. BABCOCK,[1] CAPT. JOSEPH N. STABILE, SnC., USA, ERNEST WINDHAM, LARUE B. EVANS, AND COL. RAYMOND RANDALL, VC, USA.
Veterinary Division, Army Medical Department Research and Graduate School, Army Medical Center, Washington, D. C.

Earlier studies (2, 6, 7, 9, 10) have shown that freezing and storage temperatures affect the physical character of homogenized milk. Previous studies (3, 4, 8) also have shown that when homogenized milk was frozen, the solid components tended to concentrate in the lower portion of the sample during the freezing process; apparently there was no further movement of these solids after the milk was frozen. A more recent study (5) has shown that frozen homogenized milk of good quality can be stored at usual storage temperatures after thawing without deterioration for longer periods of time than usually are necessary before use.

The literature does not contain information regarding the effect of the age of homogenized milk before freezing on its keeping quality after freezing. The present study was undertaken to determine the effect of age before freezing on the keeping quality of frozen homogenized milk.

PROCEDURE

Homogenized milk samples with a fat content of 3.8 per cent packaged in one-half pint paper containers by a commercial dairy in Washington, D. C., were used. The milk had been pasteurized at 155° F. for 30 minutes. Fifty-six samples were taken directly from the filler and divided into seven groups of eight samples each. One sample was examined immediately for flavor, bacterial count, coliform organisms, titratable acidity, pH, and sediment and the remaining seven samples of this group were placed in a freezer held at – 17.5° C.[2] The remaining samples were held at 1.67° C. Eight samples, each representing a separate group, were removed from the 1.67° C. storage after 12, 24, 48, 72, 96 and 120 hours. Each time, one of the eight samples of the respective group was used for laboratory examination and the remaining seven were placed in a freezer at – 17.5° C. The samples were removed from the – 17.5° C. storage and thawed for laboratory examination after having been held in the frozen state for 5, 28, 38, 47, 56, 76, and 86 days. The determinations for bacterial content,

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[1] Formerly Lieutenant Colonel, SnC., AUS. Present address: Dairy Branch, Production and Marketing Administration, U. S. Department of Agriculture.

[2] The thermostat controlling the temperature of this freezer had sufficient lag to cause a temperature variation of about eight degrees.

TABLE 1

Effect of age before freezing on the keeping quality of frozen homogenized milk

Age before freezing (Hr.)	Not frozen	No. of days frozen						
		5	28	38	47	56	76	86
				Flavor				
0	Normal	Normal	Normal	Normal	Normal	V. Sl. ox.	Sl. ox.[a]	Sl. stale—ox.
12	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Sl. stale—ox.
24	Normal	Normal	Normal	Normal	Normal	Normal	Sl. ox.[a]	Sl. stale—ox.
48	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Stale—ox.
72	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Stale—ox.
96	Normal	Normal	Normal	Normal	.....	V. Sl. oxidized	Normal	Stale—ox.
120	Normal	Normal	Normal	Normal	Normal	Sl. oxidized	Normal	Stale—ox.
				Bacterial count[b]				
0	3600 +	1000 +	2100 +	1400 –	1600 –	2200 –	.....	4900 +
12	3400 –	1000 +	1500 –	1600 –	1600 +	2600 –	.....	2800 –
24	1500 –	1300 –	1200 –	1300 –	1800 –	1600 –	.....	2600 –
48	900 –	900 –	2500 –	1100 +	1100 –	900 –	.....	2300 –
72	700 –	600 –	1800 +	1700 –	1000 –	..... +	.....	2500 –
96	1100 –	600 –	1300 –	1200 +	1400 –	1500 –	.....	1100 –
120	1100 +	700 –	500 –	1100 –	800 –	1600 +	.....	1200 –

TABLE 1 (continued)

Effect of age before freezing on the keeping quality of frozen homogenized milk

	Sediment							
	(*ml.*)	(*ml.*)	(*ml.*)	(*ml.*)	(*ml.*)	(*ml.*)	(*ml.*)	(*ml.*)
0	0.02	0.02	0.25	0.40	0.60	1.20	1.20	4.50
12	0.03	0.02	0.10	0.60	0.25	0.70	1.30	3.50
24	0.03	0.02	0.10	0.40	0.30	1.10	2.20	3.80
48	0.02	0.02	0.55	0.20	0.50	0.60	2.70	2.50
72	0.02	0.03	0.30	0.90	0.60	1.10	2.60	4.50
96	0.02	0.02	0.40	0.50	.....	1.30	1.60	2.20
120	0.02	0.02	0.20	0.40	0.65	0.60	2.00	3.00

	Acidity															
	Titra-table	pH	Titra-table	pH	Titra-table	pH	Titra-table	pH	Titra-table	pH	Titra-table	pH	Titra-table	pH	Titra-table	pH
0	0.115	6.67	0.125	6.62	0.115	6.62	0.110	6.66	0.115	6.68	0.115	6.53	0.130	6.56	0.130	6.56
12	0.125	6.79	0.120	6.63	0.115	6.58	0.110	6.62	0.115	6.62	0.110	6.52	0.130	6.55	0.130	6.55
24	0.120	6.64	0.120	6.61	0.120	6.60	0.110	6.63	0.115	6.64	0.115	6.51	0.125	6.61	0.125	6.61
48	0.120	6.60	0.115	6.53	0.115	6.72	0.115	6.62	0.110	6.67	0.110	6.52	0.125	6.59	0.125	6.59
72	0.120	6.63	0.110	6.59	0.110	6.72	0.115	6.61	0.110	6.64	0.110	6.53	0.130	6.63	0.130	6.63
96	0.120	6.65	0.115	6.65	0.115	6.58–	0.115	6.60	.....	.....	0.110	6.53	0.130	6.62	0.130	6.62
120	0.120	6.64	0.115	6.62	0.115	6.62	0.110	6.61	0.115	6.63	0.110	6.60	0.130	6.62	0.130	6.42

[a] ox. = oxidised

[b] + and – denote presence or absence of coliform organisms in 1-ml. samples.

coliform organisms, titratable acidity, and pH were made in accordance with the methods outlined in Standard Methods for the Examination of Dairy Products (1). The plates were incubated at 37° C. for 48 hours. Flavor determinations were made by a panel of three men, all of whom were experienced in milk judging. The sediment was determined by the method used by the authors in their earlier studies (2, 3).

RESULTS

The effect of age before freezing on the keeping quality of frozen homogenized milk is shown in table 1. The milk was of good flavor throughout the 120 hours that it was held at 1.67° C. before freezing. The flavor of the milk remained good when it was held in the frozen state for 47 days, regardless of its age before freezing. Some of the samples that were thawed after they had been frozen for 56 days and for 76 days had developed a slight oxidized flavor. However, there was no correlation between the age of the samples before freezing and the development of this flavor. When the samples were thawed after holding in the frozen state for 86 days, they had a stale and oxidized flavor. There was an insignificant tendency for these flavors to be more pronounced as the age of the samples before freezing was increased.

Table 1 also shows that there was no significant change in the bacterial content of the samples either in the 120 hours that they were held at 1.67° C. or in the 89 days that they were held in the frozen state at −17.5° C. There apparently was a slight coliform contamination of the milk that was used in the preparation of the samples. With the exception of those samples which were held at 1.67° C. for 24 hours before freezing, at least one sample in each age group was positive for coliform organisms in 1-ml. portions either before or after freezing. Two of the samples before freezing and two each of those held in the frozen state for 5, 28, 47 and 56 days gave positive coliform tests. Of these samples held in the frozen state for 47 and for 86 days, one each gave a positive test. There was no correlation between the positive coliform tests in the samples before and after freezing.

Table 1 shows further that the quantity of sediment remained low and constant in the milk samples before they were frozen. It did not increase when the samples were held in the frozen state for 5 days. When held for 38 days, however, the quantity of sediment had materially increased and it continued gradually to increase as the storage time lengthened. Significant separation, as shown by the sediment readings, had occurred when the samples were thawed after they had been held for 47 days in the frozen state, and considerable sediment was present in all the samples when they were thawed after having been frozen for 89 days. There was, how-

ever, no correlation between the degree of separation and the age of the sample prior to freezing.

The acidity as shown by titration and by pH determinations did not vary significantly either before the samples were frozen or while they were held in the frozen state. The titratable acidity ranged from 0.110 to 0.130 per cent and the pH values from 6.51 to 6.79. These variations were well within the limits of experimental error. The titratable acidity values were lower than usually encountered because of the dilution technic employed.

CONCLUSION

It is recognized that homogenized milk should be frozen as soon as possible after processing, but if the milk is of good quality it may be kept as long as 120 hours at 1.67° C. before freezing without adversely affecting the keeping quality of the frozen product.

The authors wish to express their appreciation to Edith Giltner and Elmina Dickson for their assistance with the analytical determinations.

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FERTILITY LEVEL OF BULL SEMEN DILUTED AT 1:400 WITH AND WITHOUT SULFANILAMIDE

G. W. SALISBURY[1] AND R. W. BRATTON

Laboratory of Animal Breeding and Artificial Insemination, Department of Animal Husbandry, Cornell University, Ithaca, New York

The basic advantage of artificial insemination, the ability to breed more cows to superior sires than can be done in natural service, can be realized on a broad practical basis only if the single ejaculate of a superior bull can be divided and many cows bred with it. The practical difficulty involved in inseminating extremely small volumes of semen has resolved the problem to standardizing the volume inseminated at 1.0 ml. and diluting the semen with appropriate amounts of diluter. This was done to reduce the spermatozoa numbers to a level consistent with maximum efficiency of use of semen and optimum fertility.

Earlier studies (3, 4, 5) have shown that bull semen may be diluted at levels as high as 1 part of semen to 100 parts of the yolk-citrate diluter with no detectable effect on the fertility level. The present report deals with experiments designed to test even wider dilution rates.

EXPERIMENTAL PROCEDURE

The investigations reported here were conducted in cooperation with the New York Artificial Breeders' Cooperative, Inc. The general methods used in handling the semen and the methods used in determining the results of each insemination have been reported earlier (3, 4, 5). Two investigations were made. The first was to study the effect of levels of dilution above 1:100 when the yolk-citrate diluter was used. This diluter was composed of equal parts fresh egg yolk and a buffer composed of 3.6 g. of sodium citrate dihydrate per 100 ml. of water distilled in glass and autoclaved for 20 minutes at 15 lb. pressure.

Preliminary evidence suggested that some decrease in fertility might be expected at dilution rates above 1:100. Therefore, a carefully designed experiment was conducted using the semen of one bull of consistently high fertility. The experiment was in the form of a 4×4 Latin square, each of four ejaculates being split into four aliquots and each of these aliquots being diluted at rates of one part of semen to 100, 200, 400 and 800 parts of the yolk-citrate diluter. It was impossible for each of the 75 different inseminators involved in this study to use semen diluted at each level for each of the ejaculates used. Therefore, the inseminators were divided arbitrarily into four groups. Each group received semen diluted at one

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[1] Now at Department of Dairy Production, University of Illinois, Urbana.

single rate for one particular collection, but each group received all dilution rates at some time during the experiment. Such a design, although somewhat cumbersome to manipulate in the central headquarters from which the semen was shipped, presented no difficulty in the field and permitted precise measurements of the results.

The second experiment was with 19 different bulls and was designed as a randomized block experiment. Each bull was considered as a block. Five collections were made from each bull with intervals of approximately 10 days between collections. Dilution rates were assigned at random to these collections. Each semen collection was diluted at one rate, i.e., one part of semen to 100, 150, 200, 300 or 400 parts of the diluter used. The semen was shipped to all inseminators using semen of that particular breed. Four breeds were represented. The diluter used was composed of equal parts egg yolk and a sodium citrate-sulfanilamide buffer consisting of 3.6 g. sodium citrate dihydrate and 0.6 g. sulfanilamide made up to 100 ml. with water distilled in glass. The final concentration of sulfanilamide in the diluter was 300 mg. per 100 ml. This experiment, although not giving as precise estimates of the sources of random variation as the first, was much more extensive in scope, and more general conclusions and recommendations for practice could be drawn from it.

A statistical study also was made of the relation between the number of spermatozoa inseminated and fertility level in the case of approximately 700 ejaculates of semen routinely used in artificial insemination between January 1 and August 31, 1947.

RESULTS

In the first experiment the average numbers of spermatozoa in 1 ml. of diluted semen at each dilution rate were: for 1:100, 14,580,000; for 1:200, 7,330,000; for 1:400, 3,670,000 and for the 1:800 dilution rate, 1,840,000 spermatozoa per 1 ml. inseminated. The results are presented in table 1 and include services to cows bred for the first and for the second time in a service period. An analysis of covariance using services as the independent and 165-day (5-month) non-returns as the dependent variable showed that the differences observed were significant, the 1:400 and 1:800 rates being different from the 1:100 rate. Also, it should be noted that the cows bred with the semen diluted at 1:100 apparently held to service better than did the cows bred with semen diluted at the higher rates. This fact was of considerable interest and prompted the second experiment.

In the second experiment the average number of spermatozoa in 1 ml. of the diluted semen was: for 1:100, 12,060,000; 1:150, 8,490,000; 1:200, 6,340,000; 1:300, 4,160,000 and for the 1:400 dilution rate, 3,290,000 spermatozoa. The means of the semen quality characteristics are presented in table 2. Statistical analyses of these data show that none of the differ-

TABLE 1

Fertility of the semen of one bull diluted at various levels with yolk-citrate, 45, 75 and 165 days after insemination

	Ratio of semen to diluter				
	1: 100	1: 200	1: 400	1: 800	Total
Services	98	106	82	70	356
% non-returns					
Av. 45 days	76.5	63.2	56.1	61.4	64.6
Av. 75 days	67.3	50.9	45.1	47.1	53.4
Av. 165 days	61.2	48.1	36.6	44.3	48.3

ences were significant. Thus, it is concluded that the design did not bias the experiment towards different qualities of semen for use in preparation of different dilutions.

The results of the inseminations are shown in table 3. They represent only those services to cows being bred for the first time in a service period and are given as 45-day (1 month), 75-day (2 month) and 165-day (5 month) non-returns to service. Statistical analysis of the data indicated that the mean differences in fertility level were not statistically significant. In fact, the variance due to treatments from the analysis of covariance and that due to random variation in the data were almost identical, the F value being 1.01 for 75-day non-returns and 0.78 for 165-day non-returns. However, a trend toward a decrease in fertility with each decrease in the number of spermatozoa inseminated is apparent in the mean values for each dilution rate given in table 3. The correlation between the number of spermatozoa inseminated and the fertility of each ejaculate used in the experiment was 0.24, a small but significant figure at the 5 per cent level of probability. The relationship indicated by the correlation coefficient explained but a minor portion of the variance in fertility level observed. The regression of fertility level on spermatozoa numbers inseminated was linear. Between the limits of numbers of spermatozoa per insemination used, the calculated regression formula was $Y = 51.27 + 0.777X$, where Y = estimated per cent of 165-day non-returns and X = the number of spermatozoa inseminated. This

TABLE 2

Average of the semen quality characteristics for each dilution rate

	Ratio of semen to diluter				
	1: 100	1: 150	1: 200	1: 300	1: 400
Initial motility, %	72.1	71.8	71.3	71.8	71.1
Concentration, 1,000's/mm.^{3}	1,218	1,282	1,274	1,253	1,318
Methylene blue reduction time, min.	5.0	4.9	4.7	4.9	4.6

result is equivalent to a change in fertility level of approximately 0.8 per cent for each change of one million spermatozoa between the limits used in the experiment.

Similar calculations for approximately 700 ejaculates used routinely in artificial insemination resulted in a smaller but a statistically significant regression coefficient. The regression equation was $Y = 56.86 + 0.3146X$ or equivalent to a change of approximately 0.3 per cent in fertility for each change of one million spermatozoa inseminated. However, the regression calculated on this latter data was for a different range of spermatozoa numbers inseminated than was the case of the experimental data. The extreme ranges of spermatozoa numbers inseminated in the routine work were from 6,700,000 to 34,600,000 per insemination, with the mean being 14,700,000 spermatozoa. In contrast, the range in the last experiment was from 2,360,000 to 15,300,000 spermatozoa per insemination.

TABLE 3

Fertility of the semen of 19 bulls diluted at various rates with yolk-citrate sulfanilamide, 45, 75 and 165 days after insemination

	Ratio of semen to diluter					
	1: 100	1: 150	1: 200	1: 300	1: 400	Total
Total services	1408	1580	1581	1379	1395	7343
% non-returns						
Av. 45 days	69.1	64.3	66.7	62.6	62.6	65.1
Av. 75 days	60.9	56.3	58.4	55.0	52.8	56.7
Av. 165 days	58.1	53.2	55.4	51.3	48.5	53.3

DISCUSSION

This series of investigations to determine the optimum dilution rate for fertile bull semen and the minimum number of bovine spermatozoa required for maintenance of optimum fertility in artificial insemination appears to present a number of problems, some of which have been answered only partially. In the first place, the data reported earlier (3, 4, 5) showed no decrease in fertility as the numbers of spermatozoa inseminated were decreased from approximately 400 million down to approximately 13 million spermatozoa per insemination. In those experiments, yolk-citrate diluter, made up of equal parts of fresh egg-yolk and a solution containing 3.6 g. or slightly more of sodium citrate dihydrate per 100 ml. of water distilled in glass was used.

When the numbers of spermatozoa were reduced further by increased dilution with the same diluter used in the first experiment reported here, the decrease in fertility noted was large and the differences were significant. This fact suggested that the minimum number of spermatozoa per insemination for optimum fertility had been reached. In contrast, however, the second experiment, in which sulfanilamide was added to the diluter, failed

to show the same great decrease in fertility level over a range of spermatozoa numbers similar to that used in the first experiment. A downward trend in fertility was suggested by the regression calculation. An even smaller downward trend in fertility with decreasing spermatozoa numbers was shown for semen used in routine artificial insemination work in which the yolk-citrate sulfanilamide diluter was regularly used.

These facts suggest that the curve of declining fertility with decreasing spermatozoa numbers probably is a logarithmic one in which the plateau of approach to the optimum is long and the slope very small. However, as the minimum is approached, the rate of decrease in fertility accelerates, the slope of the curve becomes greater and the fertility level probably reaches zero before spermatozoa numbers reach that level.

Secondly, the data presented here and other evidence from this laboratory suggest that the position of the curve, although perhaps not its slope, may be altered to some degree by the diluter in which the spermatozoa are suspended. It has been shown that the livability of bull spermatozoa is shortened by greater dilutions (4). Also, it has been shown that bull spermatozoa in low concentrations are harmed by oxygen (2). Sulfanilamide depresses the oxygen consumption by spermatozoa, stimulates increased livability (1), and improves the fertility of spermatozoa used for insemination after storage (6). It is not known that sulfanilamide will increase the inherent fertility of bull semen if that semen were used for insemination immediately after collection. Rather, the effect in increasing fertility earlier observed (6) is believed to be due to prolongation of innate fertilizing capacity rather than an actual increase in the potential.

Based on these observations it is suggested that the addition of sulfanilamide to the diluter in the last experiment reported here preserved the life of the spermatozoa in low concentrations better than was done by the yolk-citrate. Thus, the downward acceleration of the fertility level was partially prevented at the levels of spermatozoa numbers used. However, it is believed that with somewhat lower numbers of spermatozoa, the accelerated decrease in fertility would be observed. Until more fundamental information is available leading to control of the metabolic processes involved, it appears that the minimum number of spermatozoa from fertile bulls which should be used for insemination of cows rests at between 5 and 10 millions per insemination. In the case of particularly valuable proved sires that are highly fertile, lower numbers of spermatozoa can be used but a sacrifice in fertility level probably would result.

Finally, it should be emphasized that these experiments were carried out under field conditions in which shipment of semen was routine. Most inseminations were made the second, third and fourth days after the semen was collected. The semen was from normal bulls of high fertility. It was of excellent quality, as shown in table 2. However, these experiments do not

enable one to speculate on the probable fertility of highly diluted semen from bulls of low fertility. Nor do the minimum numbers mentioned above imply that bulls producing semen of low spermatozoa count will be fertile.

SUMMARY

In one investigation it was found that the practical limit of dilution rate was about 1:100 when the yolk-citrate diluter was used. In another experiment involving 7,343 inseminations when sulfanilamide was added to the yolk-citrate diluter at the rate of 300 mg. per 100 ml., no difference was found in fertility level between dilution rates of one part of semen to 100, 150, 200, 300 and 400 parts of the yolk-citrate-sulfanilamide diluter.

However, a trend downwards amounting to 0.8 per cent in fertility level for each decrease of 1 million spermatozoa inseminated was observed, over the range of 2.36 to 15.30 millions of spermatozoa inseminated. The probable reasons for the different results of the two experiments are discussed.

With present handling and insemination techniques, it is suggested that the minimum number of spermatozoa consistent with optimum fertility rests at 5 to 10 millions from bulls of known fertility.

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AN ANALYSIS OF THE RESULTS OF THE 1947 COLLEGIATE STUDENTS' INTERNATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

G. M. TROUT, E. O. ANDERSON, C. J. BABCOCK, P. A. DOWNS, AND F. H. HERZER

Committee on Judging Dairy Products, American Dairy Science Association

A few items on the score cards used in the Collegiate Students' International Contest in the Judging of Dairy Products frequently have been questioned as to their real values in testing the judging abilities of the contestants. The inference sometimes is made that certain items are scored as effectively by the lower ranking contestants as by the higher ranking contestants. If this is true, these items may have little weight in determining the judging abilities of contestants. Some coaches of dairy products judging teams have felt that high ranking contestants attain their standing, in part at least, because of extreme conservatism in certain phases of scoring.

The contestants' score cards from the 1947 contest were made available to determine if high standings were attained without actually showing proficiency in judging. Also, it seemed desirable to determine if any technics were employed by the winning contestants that could be used in the training of judging teams.

PROCEDURE

The data included herein were obtained from a study of the score cards of 57 contestants. Each scored ten samples of creamery butter, milk, Cheddar cheese and vanilla ice cream. Hereafter, these products will be designated as butter, milk, cheese and ice cream. Two hundred and twenty-eight contestant cards were examined and 2,280 judgments were involved.

The contestants' score cards for butter, milk, cheese and ice cream were grouped into quartiles. The first quartile consisted of the cards of the 14 contestants scoring highest in the judging of that specific product; the second quartile contained the cards of the 14 next highest contestants, and so on. The quartiles for the different products may or may not have represented the same contestants. It should be pointed out that the fourth quartile included cards from contestants who through carelessness failed by omission or commission to score properly the various items and, therefore, were given the maximum penalty. For this reason the data obtained from the cards in the fourth quartile may not be significant.

DISCUSSION OF THE DATA

An examination of the data presented in tables 1 to 4, inclusive, reveals

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DISCUSSION OF THE DATA

An examination of the data presented in tables 1 to 4, inclusive, reveals

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TABLE 1

The grades (points lost) of the three high individuals and quartiles of contestants in the judging of butter

Class	Points lost in scoring								Total
	Flavor		Body & texture		Color		Salt		
	Score	Criticism	Score	Criticism	Score	Criticism	Score	Criticism	
1st individual	7.00	1.00	0.0	0.0	0.0	0.0	0	0	8.00
2nd "	7.00	2.50	0.0	0.0	0.0	0.0	0	0	9.50
3rd "	9.00	2.00	0.0	0.0	0.0	0.0	0	0	11.00
Av. of 3 high individuals	7.66	1.83	0.0	0.0	0.0	0.0	0	0	9.49
1st quartile	9.04	3.25	0.29	0.43	0.0	0.0	0	0	13.01
2nd "	11.39	3.93	0.96	1.43	0.0	0.0	0	0	17.71
3rd "	12.00	3.71	2.14	1.29	0.11	0.07	0	0	19.32
4th "	16.43	5.20	3.67	3.67	0.0	0.0	0	0	28.97

information very similar to that noted in previous studies (1, 2); *i.e.*, the high ranking contestants in each product attained that position because they knew how to score properly the "flavor" and "body and texture."

The following deductions from the data may be helpful:

Flavor. The three high contestants in the scoring of the specific products were not significantly better than the others in criticizing the flavor of cheese, ice cream and milk. However, the three high individuals showed superior ability to describe and evaluate the flavor of butter. Generally all contestants lost more points in placing a score on flavor than they did in recognizing a flavor.

Body and texture. In cheese judging, the two high individuals surpassed their close competitors both in recognizing defects of body and tex-

TABLE 2

The grades (points lost) of the three high individuals and quartiles in the judging of Cheddar cheese

Class	Points lost in scoring						Total
	Flavor		Body & texture		Color		
	Score	Criticism	Score	Criticism	Score	Criticism	
1st individual	9.00	6.00	4.50	1.50	1.00	2.00	24.00
2nd "	9.00	5.50	4.00	3.00	1.50	3.00	26.00
3rd "	10.50	6.50	7.00	3.50	1.00	2.00	30.50
Av. of 3 high individuals	9.50	6.00	5.16	2.66	1.16	2.33	26.81
1st quartile	11.18	6.14	7.39	4.04	1.14	2.14	32.03
2nd "	13.89	7.18	7.86	5.79	1.39	2.57	38.68
3rd "	15.04	7.00	11.64	5.96	1.18	2.36	43.18
4th "	20.10	7.57	12.50	6.60	2.10[a]	3.10	51.97

[a] Only 13 contestants involved. Data from two contestants were so abnormally out of line they were not included.

TABLE 3

The grades (points lost) of the three high individuals and quartiles in the judging of milk

Class	Points lost in scoring					Total
	Flavor		Sediment	Container & closure		
	Score	Criticism	Score	Score	Criticism	
1st individual	8.50	6.00	3.00	0.50	0.50	18.50
2nd "	18.00	4.00	2.25	0.25	1.00	25.50
3rd "	12.00	6.50	2.50	2.25	3.50	26.75
Av. of 3 high individuals	12.83	5.50	2.58	1.00	1.66	23.57
1st quartile	16.50	5.62	4.08	2.09	1.99	30.28
2nd "	20.96	6.51	4.68	1.48	2.42	36.05
3rd "	25.14	6.35	6.17	1.96	2.99	42.61
4th "	30.30	7.08	8.11	2.55	3.03	51.07

ture and in weighing the criticism. In body and texture of butter, the first three individuals agreed precisely with the judge and the contestants in the first quartile did not vary too widely from the judge. However, in criticizing ice cream, the three high contestants were not significantly better than those in the first quartile; in evaluating the criticism, the two highest individuals were somewhat superior to their immediate competitors.

Miscellaneous items. Neither the judges nor the contestants criticized the salt content in butter in this contest. However, some contestants criticized the color of butter, but the judge did not do so.

In the judging of the color of cheese, the three high individuals did no better than did those in the first three quartiles.

Practically all contestants scored and criticized similarly the melting quality of ice cream. However, the scoring of color of ice cream was evidently a "booby trap" for some of the contestants, especially those in the fourth quartile.

TABLE 4

The grades (points lost) of the three high individuals and quartiles in the judging of vanilla ice cream

Class	Points lost in scoring								Final grade
	Flavor		Body & texture		Melting quality		Color		
	Score	Criticism	Score	Criticism	Score	Criticism	Score	Criticism	
1st individual	12.00	5.70	3.50	2.50	2.50	3.00	0.0	0.0	29.20
2nd "	15.50	6.50	4.50	2.50	1.50	1.00	0.0	0.0	31.50
3rd "	16.00	7.50	6.00	2.00	0.50	0.00	0.0	0.0	32.00
Av. of 3 high individuals	14.50	6.57	4.66	2.33	1.50	1.33	0.0	0.0	30.89
1st quartile	16.17	6.05	5.79	2.79	1.93	2.04	0.04	0.07	34.88
2nd "	17.39	6.34	7.93	4.76	2.14	2.64	0.32	0.14	41.66
3rd "	22.04	7.32	9.38	5.26	2.29	2.36	0.29	0.29	49.23
4th "	21.57	7.52	10.50	7.33	2.23	2.30	4.30	0.33	56.08

In scoring sediment of milk, it is possible that the three high individuals retained a more accurate mental picture of the sediment standards than other contestants and thereby attained a better score. The item "container and closure" of milk evidently had been so well impressed upon the minds of all contestants that little variation existed in the scoring and criticizing of this item.

The fourth quartile. Table 5 was compiled to demonstrate in what area the contestants of the fourth quartile failed most seriously. The data were secured by subtracting the average grade of the first quartile from that of the fourth quartile. The data seem to indicate that the chief weakness of the contestants represented in the fourth quartile was their inability to place a correct score on the flavor of the product being judged. In criticizing the flavor of the products, there was little difference between contestants of the first and fourth quartiles. This is indicated by the fact

TABLE 5

Difference in scores between 1st and 4th quartiles

Product	Score card items										
	Flavor		Body & texture		Color		Sediment	Container & closure		Melting quality	
	Score	Criticism	Score	Criticism	Score	Criticism	Score	Score	Criticism	Score	Criticism
Butter	7.39	1.95	3.39	3.25	.....	.....	.....	.....	.....	.....	.....
Cheese	8.92	1.43	5.11	2.56	1.06	0.96	.....	.....	.....	.....	.....
Milk	13.80	1.46	.....	.....	.....	.....	4.03	0.46	1.04	.....	.....
Ice cream	4.87	1.47	4.71	4.54	4.27	0.26	.....	.....	.....	0.30	0.26

that the fourth quartile lags behind only 1.95 points in butter, 1.43 points in cheese, 1.46 points in milk and 1.47 points in ice cream. A marked difference existed between the first and fourth quartiles when the sense of touch (body and texture criticisms) was involved. This is shown by the divergence of the scores of body and texture in butter, cheese and ice cream. Contestants of the fourth quartile lost as many points in scoring body and texture of ice cream as they did in scoring flavor. Likewise, they lost heavily in the scoring of body and texture in cheese. The ratio between the criticism of body and texture and its score is narrow. From the data available it is not possible to make a precise observation on this point. The solution to the difficulty may lie in devoting more time to the development of the sense of touch.

Effect of range of score on contestant rating. It has been alleged that contestants, when in doubt as to the correct score, tend to judge an item conservatively. Official judges sometimes are criticized similarly. Data in table 6 indicate that official judges used about 75, 57, 63 and 89 per cent of

TABLE 6

Range in scores of officials and contestants in scoring flavor of dairy products

Class	Range of score used in scoring flavor of							
	Butter (8)[a]		Cheese (7)[a]		Milk (15)[a]		Ice cream (9)[a]	
	Range	%	Range	%	Range	%	Range	%
Official	6.0	75.0	4.0	57.1	9.5	63.3	8.0	88.9
1st individual	5.0	62.5	4.0	57.1	15.0	100.0	3.5	38.9
2nd "	5.0	62.5	4.5	64.3	6.5	43.3	3.5	38.9
3rd "	5.0	62.5	5.0	71.4	10.0	66.7	3.0	33.3
1st quartile	5.5	68.8	4.5	64.3	9.9	66.0	4.4	48.9
2nd "	5.2	65.0	3.8	54.3	9.6	64.0	4.2	46.7
3rd "	5.2	65.0	3.6	51.4	8.0	53.3	5.0	55.5
4th "	5.3	66.3	5.3	75.7	9.4	62.7	4.7	52.2

[a] Normal range in flavor score for the product.

the normal range in scoring the flavor of butter, cheese, milk and ice cream, respectively.

In the scoring of butter for flavor, contestants of the first quartile used a slightly greater percentage of the normal range than did contestants of the remaining quartiles. The three highest individuals used a very slightly lower proportion of the normal range than did the average of the four quartiles.

The official judge of cheese was more conservative in the use of the score range of flavor than the judges of the other products. The ranking individual used the same range. Contestants making up the second and third quartiles were even more conservative. On the other hand, individuals composing the fourth quartile used about 76 per cent of the recommended range.

TABLE 7

Range in scores of officials and contestants in scoring body and texture of cheese and ice cream

Class	Range of score used in scoring body and texture of			
	Cheese (3.5)[a]		Ice cream (4.5)[a]	
	Actual range	% of normal range	Actual range	% of normal range
Official	3.5	100.0	2.0	44.4
1st individual	2.0	57.1	1.5	33.3
2nd "	2.5	71.4	1.5	33.3
3rd "	3.0	85.7	1.0	22.2
1st quartile	2.2	62.8	1.8	40.0
2nd "	2.3	65.7	1.6	35.5
3rd "	1.8	51.4	1.6	35.5
4th "	2.4	68.5	1.5	33.3

[a] Normal range in body and texture score for the product.

In scoring milk for flavor, most of the contestants used about the same range as the official judge. The winning contestant used the entire normal range. This contestant was not outstanding in determining the flavor defects, but he did a superior job in evaluating the flavor defect. The contestant winning second place in the scoring of milk used less than half the percentage of the normal range used by the first place winner, yet this contestant lost 10.5 more points in evaluating flavor than did the individual placing first.

All students, regardless of quartile or individual standing, seemed to be unusually moderate in the scoring of the flavor of ice cream. The official judge utilized approximately 89 per cent of the normal range. Most of the contestants used one-third to one-half of the recommended range.

Contestants were more conservative than the official judge in using the recommended range for body and texture of cheese (table 7). The official judge of cheese used 100 per cent of the normal range. The official judge and all of the contestants were extremely conservative in using the recommended range of body and texture of ice cream.

GENERAL DISCUSSION

The score cards as set up for the judging of butter, cheese, milk and ice cream contain some items which do not test proficiency in judging. This has been recognized for several years. The fact that they do not test proficiency is no indication that the items should be eliminated. For example, data show that most participants judge bottle and cap about as well as the winning contestants. Deletion of this item might result in paying too little attention to the featuring of a clean and attractive bottle. A knowledge of the proper scoring of items of lesser importance is part of a student's training and should be retained. It is hoped that the time will come in the training of students when the item of flavor, for example, may be judged with less difference between the grades of the higher and lower ranking contestants than exists at present. When that time comes, the training in dairy products judging will be more effective.

In studying the data presented, the reader should keep in mind that the products used in the 1947 Collegiate Students' International Contest in the Judging of Dairy Products may have been of such a character that utilization of the entire normal range was not feasible, because the products may not have merited different scores. It should be emphasized that in any contest the "normal range" need not necessarily be used.

SUMMARY

1. The key to the success of the winning contestants lay in their abilities to evaluate the flavor of the dairy products more nearly in line with the judgment of the official than their competitors. Also, they approximated

more nearly the judges' decision in scoring and determining the body and texture of Cheddar cheese and vanilla ice cream. The three high contestants agreed that no body and texture defects were present in the butter samples scored.

2. The items of melting quality and color of ice cream, sediment, container and closure of milk, color and salt of butter, and color of cheese are not so important as flavor and/or body and texture in testing the judging proficiency of the participants in the contest.

3. With the exception of ice cream, contestants of the fourth quartile failed to score higher because of their inability to place a correct score on the flavor of the product.

4. Concerning the use of the suggested normal range in the scoring of each product, the following tendencies were observed: In the scoring of the flavor of butter, the contestants were more conservative than the judges. In the rating of the flavor of cheese and milk, the contestants and the judges were equally moderate. In the scoring of the flavor of ice cream, the contestants were much more conservative than the judge. All contestants were exceedingly cautious in placing scores on the body and texture of cheese and ice cream.

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MANOMETRIC MEASUREMENT OF THE GAS DESORBED FROM VACUUMIZED WHOLE MILK POWDER

J. H. HETRICK[1] AND P. H. TRACY
Department of Food Technology, University of Illinois, Urbana

Packaging whole milk powder in an atmosphere extremely low in oxygen is recognized as being a safeguard in the prevention of off-flavors caused by oxidation of milk constituents. During spray drying of milk, gas is entrapped in the particles. This gas is not entirely removed by the ordinary single-stage gas-packing process. Therefore, packing milk powder in an atmosphere extremely low in oxygen becomes a problem of control of occluded oxygen, and possibly adsorbed oxygen, as well as one of proper technic in gas packing.

The origin of the entrapped gas has not been established definitely. Hetrick and Tracy (3) suggest that perhaps the oxygen dissolved in the milk is a factor in the amount of oxygen entrapped in the powder, but Coulter and Jenness (1) were unable to eliminate the entrapped gas in the powder particles by deaerating the condensed milk before drying.

The composition of the entrapped gas varies somewhat with the storage time of the dried milk in an atmosphere of air, according to Haller and Holm (2). Their results showed that the gas entrapped in the particles contained from 22 to 39 per cent oxygen.

Measurement of the amount of oxygen occluded has been done in a number of ways. Lea *et al.* (4) have derived a formula by which they calculate the milliliters of oxygen per gram of powder, knowing the initial per cent oxygen and the final per cent oxygen after equilibrium has been reached in the headspace gas of nitrogen-packed powder. Haller and Holm (2) used an apparatus with which they could remove the "sorbed" gases, measure their volume, and determine their composition.

If whole milk powder is put into a container and the container evacuated, the amount of gas that is removed will depend upon the temperature of the powder, the absolute pressure, the length of time of vacuumizing, as well as the physical characteristics of the powder itself. The occluded oxygen is not removed to any appreciable extent unless vacuumizing is continued for long periods of time. Use was made of this fact in this particular study to determine the amount of oxygen or gas entrapped in the powder.

The objective of this study was to measure the rate of desorption, the amount of gas held, and the composition of the gas "desorbed" from vacuumized whole milk powder by the manometric method. The results secured

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[1] Present address: Dean Milk Company, Rockford, Illinois.

by this method also were compared with those secured by the method of Lea *et al.* (4). Using the manometric method, the effects of aging the powder in the air before vacuumizing and of saturating the milk with carbon dioxide before drying on the amount of gas held during vacuumizing, and on the composition of the desorbed gas, was determined. It was hoped that by this procedure information on the origin of the entrapped gas could be secured.

EXPERIMENTAL METHODS

The whole milk powder used in this study was prepared in the laboratory by drying preconcentrated milk in the small pilot drier. The drier was a pressure-spray type with a capacity of approximately 30 lb. of powder per hour. The apparatus shown in figure 1 was used to measure the

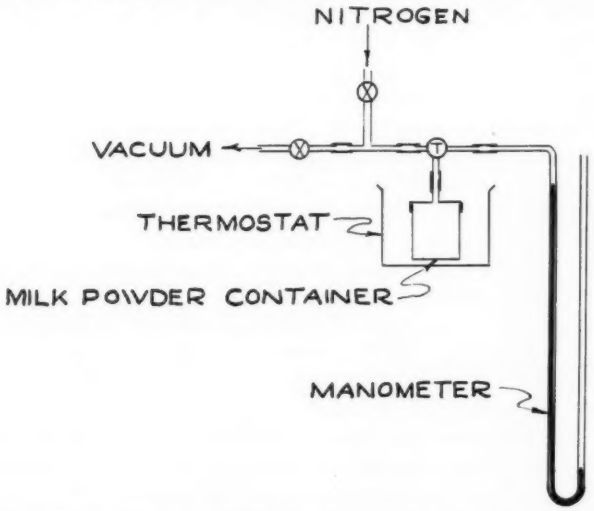

FIG. 1. Apparatus used to measure the gas desorbed from vacuumized whole milk powder.

rate of desorption and the amount of gas held. The composition of the gas desorbed from the vacuumized whole milk powder was determined by the method of Van Slyke and Sendroy (5). The whole milk powder container shown in the figure was calibrated up to the stopcock so that the packing density could be determined when the weight of milk powder was known. Capillary tubing was used for the connections and also for the manometer.

Whole milk powder was weighed into the cans so that the packing density in grams per milliliter of container volume was approximately 0.5. The manometer was affixed to the can and a three-way valve was set in such a way that the vacuum pump and the manometer both were connected

to the can of powder. By means of the vacuum pump, a vacuum was drawn on the system in approximately two minutes to an absolute pressure of 1–2 mm. mercury. The three-way cock then was turned in such a way that the can of powder was opened only to the manometer, and the vacuum pump was removed. As the entrapped air desorbed from the powder, the pressure increased. Absolute pressure measurements were made until the pressure became constant. All pressure measurements were made at 25° C. When the pressure measurements became constant, it was assumed that equilibrium conditions had been reached. Knowing the equilibrium pressure, the volume of the can, the weight of powder, and using a value of 1.31 for the density of air-free powder, the number of moles of gas, or volume of gas (*N.T.P.*) per gram of powder, which was held during the initial vacuumizing process, could be calculated. For this purpose, the following formula was developed.

$$\text{cc. gas/g.} = \left(\frac{22{,}400\ P_e}{W\ R\ T}\right) \times \left(V_c - \frac{W}{1.31}\right)$$

Where P_e = the equilibrium pressure (mm. Hg)
W = " weight of powder in grams
V_c = " volume of the container (cc.)
T = " absolute temperature
R = " gas constant (62,400 cc. mm.)
1.31 = " assumed density of air-free milk powder in grams/cc.

When the equilibrium pressure was reached, the volume of gas was so small that a sample could not be removed for analysis. In order to determine the composition of the gas, nitrogen was admitted rapidly to a known pressure and then a sample of the mixed gas was removed for analysis. Blank determinations were made in which there was no milk powder in the can to determine the per cent oxygen in the nitrogen used. Knowing the per cent oxygen in the nitrogen used, the pressure of the nitrogen, the equilibrium pressure of the gas in the container, and the total pressure and the per cent oxygen of the gas in the container after the nitrogen was admitted, the percentage of oxygen in the gas that was desorbed from the sample of milk powder could be calculated by means of the following formula:

$$X_e = \frac{(X_T P_T) - (X_N P_N)}{P_e}$$

Where X_e = the per cent oxygen in the "desorbed" gas
X_T = the per cent oxygen in the mixed gas
P_T = the total pressure (mm. Hg)
X_N = the per cent oxygen in the nitrogen
P_N = the nitrogen pressure (mm. Hg)
P_e = the equilibrium pressure (mm. Hg)

It is recognized that this method is not an absolute one for measuring the amount of oxygen held by the particles during the vacuumizing process.

It does not include the adsorbed gas which may be present under equilibrium conditions. However, it is believed to show differences among different treatments of milk powder with respect to the amount of oxygen held during vacuumization.

RESULTS

Reproducibility of the method. In order to ascertain how closely values from a number of runs would agree, four determinations on the same powder were made. The results are shown in table 1. The absolute pressure measurements were reproduced closely. The same milk powder used

TABLE 1

Reproducibility of pressure measurements during desorption of gas from vacuumized whole milk powder

Time after vacuumizing	Absolute pressure ($P_B - P$) at 25° C. (mm. Hg)			
	Trial 1	Trial 2	Trial 3	Trial 4
(*hr.*)				
0	1	1	2	2
18	21	21	21	20
27	28	28	28	28
42	30	31	30	30
55	32	31	31	31
71	34	33	33	33
90	35	34	34	34
98	35	35	35	35
114	35	35	35	36
144	36	35	36	36
187	37	36	37	37[a]
258	37	36	40	62
336	40	41	40	10
403	41	42	42	11
474	41	41	41	11

[a] Leaker re-evacuated to 2 mm.

in this experiment also was packed in nitrogen gas and gas analyses were run on the headspace gas of the samples of powder until equilibrium was reached. The formula developed by Lea *et al.* (4) was used to calculate the milliliters of oxygen per gram of powder, as well as the milliliters of gas per gram of powder, and the values were compared to those secured by the manometric method. The results are listed in table 2.

From these results it can be seen that the two methods of determination check closely with respect to the milliliters of oxygen per gram, but not so well for the milliliters of gas per gram. The conclusion to be reached is that perhaps the difficulty lies in the composition of the gas desorbed with respect to per cent oxygen. Lea *et al.* (4) have assumed in their derivation that the gas entrapped in the particles contains 20.85 per cent oxygen. If the gas desorbed has a higher oxygen content, as data by the manometric method show, then one would expect to have a lower value for the milli-

liters of gas evolved per gram of powder. The assumption that the composition of the gas entrapped in the powder is 20.85 per cent oxygen is not significant when one uses this assumption to calculate the milliliters of oxygen per gram of powder. With the formula that they have derived, any error involved in this assumption would largely cancel out. However, in the calculation of the milliliters of gas held per gram, the value for the per cent oxygen in the gas desorbed becomes important.

With the manometric method, the values for the milliliters of gas per gram are more accurate than those representing the milliliters of oxygen per gram because the pressure can be measured accurately, but small variations in the per cent oxygen in the mixed gas make large errors in the

TABLE 2

A comparison of the manometric method and the method of Lea et al. (4) for determination of ml. of oxygen and ml. gas entrapped per gram of whole milk powder

Manometric method									
Trial	Vol.	W	P_e	P_N	P_T	$\%0_2$ (P_T)	$\%0_2$ gas desorbed	Ml. gas/g.	Ml. 0_2/g.
1	477	238.5	43	712	755	2.21	33.8	0.064	0.0216
2	474	237.0	43	710	753	2.38	36.7	0.064	0.0235

Method of Lea *et al.*						
Time	% O_2	Equilibrium % O_2	D	d	ml. gas/g.	ml. O_2/g.
(*days*)						
0	0.3	.....	.....	.....	.....	.....
4	2.01	2.19	1.31	0.50	0.113	0.0235
7	2.24	.....	.....	.....	.....	.....
14	2.16	.....	.....	.....	.....	.....
26	2.33	.....	.....	.....	.....	.....

milliliters of oxygen. A difference of approximately 0.1 per cent oxygen in the gas mixture makes a difference of about 1.8 per cent in the oxygen content of the desorbed gas. However, the data are sufficiently accurate to show that the per cent oxygen in the desorbed gas is higher than that of ordinary air. This is in agreement with the results of Haller and Holm (2). One could speculate from this that the source of the entrapped air in the particles may be the gas that is dissolved in the milk before drying, or that oxygen is preferentially held in the cavities of the particle during vacuumizing.

Importance of gas entrapped in milk at time of drying. If the origin of the gas which is entrapped in the milk powder particles is the milk before drying, it was reasoned that the composition of the desorbed gas could be changed by changing the composition of the gas dissolved in milk. Two lots of powder were dried, one serving as a control and one in which

the carbon dioxide was bubbled into the condensed milk before drying. Both lots were spray dried as nearly alike as possible as far as pressures, temperatures, and nozzle sizes were concerned. Duplicate tests were run on both lots of powder when the powder was freshly prepared, and another set of duplicate runs was made after the powder was exposed to air for 24 hours after drying. The data for these runs are recorded in table 3

TABLE 3

Effect of saturating the condensed milk with carbon dioxide and age of powder before vacuumizing on the gas content and composition of the gases desorbed from vacuumized whole milk powder

Sample no.	Trial	Treatment	Vol.[a]	W	P_e	P_N	P_T	% O_2 (P_T)
1	A	Control, packed	477	238.5	29	721	750	1.75
	B	immediately	Leaker	.....	.....	.....	.....	.....
2	A	Control, packed	474	237	35	843	878	1.62
	B	24 hr.	475	237	35	853	888	1.54
3	A	CO_2 added to milk,	474	202	58	812	870	1.34
	B	packed immediately	479	210	57	800	857	1.20
4	A	CO_2 added to milk, packed after 24 hr.	468	200	61	819	880	2.10
			469	200	62	808	870	2.06

Sample no.	% O_2 desorbed gas	% CO_2 (P_T)	% CO_2 desorbed gas	Ml. gas/g.	Ml. O_2/g.	Ml. CO_2/g.
1	37.5	.....	.....	0.043	0.0162	.....
	.....	Leaker	.....	.....	.....	.....
2	33.4	.....	.....	0.052	0.0174	.....
	32.1	.....	.....	0.053	0.0170	.....
3	15.9	2.61	39.1	0.110	0.0175	0.0430
	13.8	2.75	41.3	0.104	0.0143	0.0429
4	26.3	2.19	31.6	0.116	0.0305	0.0367
	24.9	2.16	30.4	0.118	0.0294	0.0359

[a] Vol. = Volume of container (cc.).
W = Weight of powder in grams.
P_e = The equilibrium pressure (mm. Hg).
P_N = Pressure of nitrogen (mm. Hg).
P_T = Total pressure (mm. Hg).

and the pressure changes by the manometric method are recorded in figure 2. The per cent oxygen in the air desorbed from the control powder again was higher than that in ordinary air. On aging the control powder before vacuumizing, the per cent oxygen in the gas desorbed decreased slightly, but the total volume of oxygen held per gram as well as the total gas per gram increased by this treatment. Similar results on the per cent oxygen in the air desorbed were secured by Haller and Holm (2). When powder is gas-packed after aging in air for 24 hours, the per cent oxygen in the headspace gas increases over that in powder gas-packed immediately after drying (1, 3). The results of this work seem to show that even

though the per cent oxygen in the desorbed gas decreases by aging the powder 24 hours before vacuumizing, the total amount of oxygen actually increases because the total amount of gas increases proportionately more than the per cent oxygen decreases.

The effect of milk and powder treatment on composition of desorbed gas and amounts of gas held by powder. When powder was vacuumized immediately after drying, the total volume of gas held per gram of powder was more than doubled by bubbling carbon dioxide into the condensed milk before drying. The composition of the desorbed gas also was altered. This would lend support to the belief that at least in part the air or gas

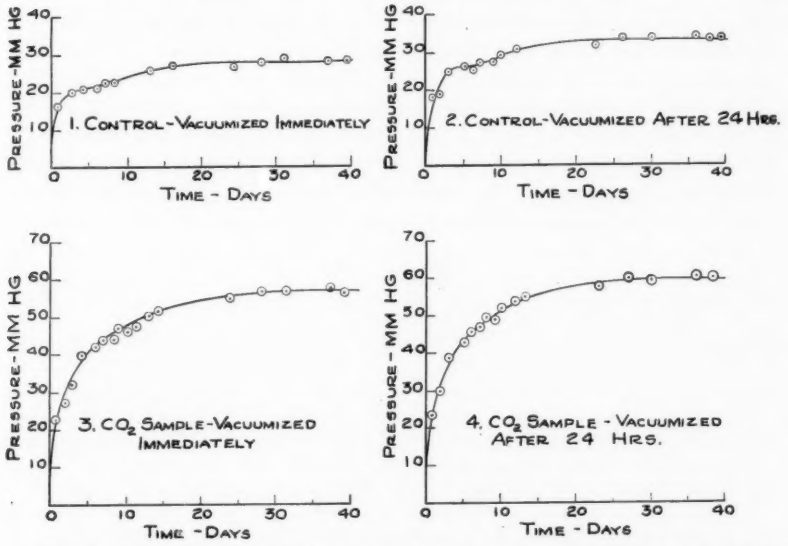
PRESSURE - MM HG
1. CONTROL - VACUUMIZED IMMEDIATELY
TIME - DAYS
2. CONTROL - VACUUMIZED AFTER 24 HRS.
3. CO_2 SAMPLE - VACUUMIZED IMMEDIATELY
4. CO_2 SAMPLE - VACUUMIZED AFTER 24 HRS.

FIG. 2. Pressure changes during desorption of gas from vacuumized whole milk powder.

dissolved in milk is a source of the gas entrapped in the powder particles. Even though the total volume of gas held per gram by this treatment was much higher than in the control powder, the volume of oxygen held per gram was nearly the same as the control batch. When powder manufactured from condensed milk that had been saturated with carbon dioxide before drying was exposed to air for 24 hours before vacuumizing, a slight increase in total gas held per gram of powder was observed and almost a two-fold increase in the amount of oxygen held per gram was found. The carbon dioxide content of the powder was decreased by exposing this powder to air for 24 hours before vacuumizing. The increase which was observed in the total gas content by exposing milk powder 24 hours before

vacuumizing could be attributed to temperature and pressure phenomena, because the powder which was vacuumized immediately was warm and as the powder cooled, it is likely that more gas would be sorbed.

SUMMARY

A manometric method is presented for the determination of the amount of gas held and the composition of the gas desorbed from vacuumized whole milk powder. The amount of sorbed gases in dried milk varied with the methods of preparation and packaging. When freshly made powder was vacuumized after drying, the per cent oxygen in the gas desorbed from powder was higher than that of ordinary air. Upon exposure of powder to air for 24 hours before vacuumizing, the per cent oxygen in the gas desorbed was less, but not lower than the per cent oxygen in normal air. The total volume of oxygen held per gram of powder was increased by exposure of powder to air for 24 hours before vacuumizing. The composition of the gas desorbed and the total quantity of gas per gram of powder could be varied by saturating the milk with carbon dioxide before drying. This would lend support to the belief that, at least in part, the source of the gas entrapped in powder is the gas dissolved in milk.

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BOOK REVIEWS

342. Advances in enzymology and related subjects of biochemistry, vol. VIII. Edited by F. F. NORD. 538 pp. $8.00. Interscience Publishers, Inc., New York, N. Y. 1948.

The 8 chapters in this volume are: Functioning of the cytoplasm; Quantitative studies on complement; Dehydropeptidases; Antifatty-liver factor of the pancreas—present status; Alkaloid biogenesis; Certain aspects of the microbiological degradation of cellulose; Synthesis of lipides; The biochemistry of fatty acid catabolism; Lipoxidase and the autoxidation of unsaturated fatty acids; and Enzymes of snake venem and their biological significance. Of the 14 authors, 5 are resident in foreign countries, thus contributing to the international flavor of the presentation. The presentation for each subject appears to be adequate and critical. The material is well-organized and well-indexed. This volume contains a cumulative index for all 8 volumes which have appeared to date. This is a valuable contribution in the struggle to summarize the increasing mass of scientific literature in forms which permit scientists to keep informed in areas outside of their limited specialties. F. E. NELSON

343. Manual of veterinary bacteriology. RAYMOND A. KELSER AND HARRY W. SCHOENING. 5th edn. 767 pp. $6.50. The Williams and Wilkins Co., Baltimore, Md. 1948.

The current edition of this book is a mixture of the up-to-date and the archaic in its field, the former predominating in some sections and the latter in others. Much material on specialized technics of value to bacteriologists handling pathogenic microorganisms, some of it not available from other common sources, is presented. The sections on general bacteriology and infection and immunity are the most in need of modernization. To mention a few specific examples, the particulate nature of bacteriophages and viruses is discussed in terms of diffusion experiments of 12–15 years ago, with no mention made of the numerous electron microscope micrographs and studies of the past 7 years. The different forms of penicillin receive no consideration. Except for two references to textbooks published in the middle thirties only one reference to work published after 1903 is given in the unexpectedly brief chapter on infection and immunity. Although the sixth edition of *Bergey's Manual* was published several months earlier

by the same publisher and the revised classification to be used had been publicized in some detail long before that, the classification employed, and thus the organization of the largest part of the book, is based upon the fifth edition of *Bergey*. In the chapter on milk and water bacteriology, bases for interpretation of results are lacking. Standards for coliform bacteria in either milk or water are not presented. Five lines, including one reference to an article published in 1935, are devoted to the phosphatase test, which might very well have occupied at least the half page devoted to the relatively unimportant color changes which bacteria may cause in milk. The illustrations are neither qualitatively nor quantitatively what one expects in a book published in 1948. The discussions on the microbiology of diseases which affect cattle are the portions which probably would be most useful to those in the dairy industry. F. E. NELSON

344. Bacteriology. A textbook of microorganisms. F. W. TANNER AND F. W. TANNER, JR. 4th edn. 625 pp. $4.50. John Wiley & Sons, Inc., New York, N. Y. 1948.

An appreciable amount of new material has been added in various places in the revision of this text, which treats bacteriology as such instead of as a branch of the medical profession. A tendency for more explanation and somewhat more concise organization than in earlier editions is apparent. The book is a good source of general information on microbiology.

Despite the generally favorable impression, several shortcomings which one hardly expects in a revision dated 1948 were encountered. The illustrations, on the whole, leave quite a bit to be desired, as many of them are either completely out of date or much inferior to what could be obtained without undue effort. By October 1947, when this book apparently went to press, the outline of the classification system used in the sixth edition of *Bergey's Manual of Determinative Bacteriology* had been publicized quite widely, so there seems little justification for retaining the outline from the fifth edition. A portion of the discussion of water bacteriology is based upon the 1933 edition of *Standard Methods for Water Analysis*.

F. E. NELSON

BACTERIOLOGY

345. Inhibition of lactic acid bacteria by analogs of pantothenic acid. WILLIAM DRELL AND M. S. DUNN. Univ. of California, Los Angeles. J. Am. Chem. Soc., 70, 6: 2057. 1948.

The growth of 23 strains of lactic acid bacteria, which require pantothenic acid, is inhibited by ω-methylpantothenic acid. The taurine analog showed less inhibitory action than the β-alanine derivative, but more than the derivative containing L-leucine. With four species of lactobacilli it was

shown that the inhibitory action of ω-methylpantothenic acid is reversed competitively by pantothenic acid over a wide range of concentrations.

H. J. PEPPLER

CHEESE

346. The manufacture of good quality cheese from pasteurized milk. H. L. WILSON, Kraft Foods Co., Chicago, Ill. Southern Dairy Products J., **44**, 1: 28–31. July, 1948.

It will not be too long before practically all Cheddar cheese produced in this country will be made from pasteurized milk. A cheesemaker has complete control of the making process when he has a good quality milk that is properly pasteurized.

The intake room must be properly supervised, every can of milk must be inspected and only milk that can be made into good quality cheese accepted. The milk should be heated just high enough and held just long enough to show a phosphatase-negative test. A standardized procedure such as is suggested in detail should be followed.

To make further progress, the cheese industry should have a program including: (a) properly constructed buildings, with up-to-date sanitary equipment kept in such condition that the milk and curd will not become contaminated; (b) a good quality of clean milk whether it is pasteurized or not; (c) a good active uniform starter; (d) approved uniform methods of manufacture; (e) curing conditions that will permit the continued activity of essential bacteria which develop the characteristic flavor of Cheddar cheese.

F. W. BENNETT

CHEMISTRY

347. Recent developments in the vitamin A field. IAN HEILBRON. J. Chem. Soc., **1948**: 386–393. March, 1948.

This material is the text of a lecture reviewing the chemistry of vitamin A, particularly from the standpoint of syntheses of the various compounds important in elucidation of structure and function. F. E. NELSON

348. Structural relationships in the natural unsaturated higher fatty acids. T. P. HILDITCH J. Chem. Soc., **1948**: 243–252. Feb., 1948.

This is the text of a lecture reviewing the structure and occurrence of these acids, particularly with respect to aquatic life and seeds.

F. E. NELSON

CONCENTRATED AND DRY MILK; BY-PRODUCTS

349. Dry milkfat as a form of storage fat. R. J. REMALEY, Kraft Foods Co., Chicago, Ill. Southern Dairy Products J., **43**, 5: 39, 46. May, 1948.

Various methods of preparation of dry milkfat have appeared in the literature. A practical method of production is described in the U. S. Patent 2,406,819 issued to Arthur W. Farrall and assigned to the Creamery Package Manufacturing Company.

Dry milkfat is produced from fresh cream. The principle involved is removal of water and serum solids from the cream by centrifugation. The cream is heated to pasteurizing temperature, preferably 170 to 190°, and separated into 80% fat. The plastic cream then is run through an homogenizer and into a continuous-type settling tank. The resulting product is approximately 98% fat. It is decanted and passed through an additional centrifuge to produce a dehydrated milkfat.

Standards of the U. S. Quartermaster Corps are: milkfat, not less than 99.8%; moisture, not more than 0.1%; copper, not more than 0.25 p.p.m.; peroxide value, zero; free fatty acids, not more than 0.5. Such dry milkfat can be stored at 40° F. for 6 months with little analytical or organoleptic change. At 0° it will remain in excellent condition for an indefinite period of time. Dry milkfat may be used any place that butter can be used.

F. W. BENNETT

FEEDS AND FEEDING

350. Preliminary observations on using a synthetic milk for raising pigs from birth. L. K. BUSTAD, W. E. HAMS, AND T. J. CUNHA, State College of Washington, Pullman. Arch. Biochem., **17**: 249. 1948.

An attempt was made to raise pigs removed from the mother at birth and placed on a synthetic milk containing all known vitamins, crude casein, a liver preparation, brewer's yeast, lactose, and colostrum substitutes. Pigs fed no colostrum substitutes (blood serum and plasma) died shortly after birth. Severe diarrhea occurred in all pigs; it could not be controlled with penicillin, sulfathalidine, sulfamethazine and Kaopectate. The obviously inadequate diet failed to keep pigs alive more than 22 days.

H. J. PEPPLER

FOOD VALUE OF DAIRY PRODUCTS

351. Sugar combined with milk in ice cream no tooth decay threat. ANONYMOUS. Ice Cream Rev. **31**, 11: 160. June, 1948.

Sugar, when combined with milk or dry skimmilk and butter, is not a

contributing cause to dental caries, according to results of a study recently conducted at the University of Wisconsin.

In experiments with cotton rats, milk was found to protect the teeth of rats against dental caries and afforded a certain degree of protection, even when the rats were fed a solid dry ration, which characteristically produced a great many carious lesions. When dry whole milk, dry skim-milk plus butter or casein-sucrose experimental diet was reconstituted with water and fed, a great reduction in dental caries was noted. It was concluded that a large part of the protective effect of milk is due to its fluidity. Other observations indicated particle size to be important in preventing tooth decay, since rations made up of very coarse sugar caused less dental decay than did rations of finely powdered sugar. W. J. CAULFIELD

ICE CREAM

352. Concentrated and dry ice cream mixes. R. J. REMALEY, Kraft Foods Co., Chicago, Ill. Southern Dairy Products J., 43, 2: 110–11. Feb., 1948.

During 1945 approximately 100,000,000 lb. of dry and concentrated ice cream mixes were shipped to the armed forces. Tests indicated that the maximum length of time they would stand up at 100° F. was 4 months. The use of ice cream mix by the housewife and small ice cream manufacturers still is increasing.

Ice cream mixes have an excellent future, provided (a) high quality dry or concentrated mix is maintained by the manufacturer; (b) the manufacturer and user rotates his product and does not extent periods of storage excessively; (c) unnecessary regulations are removed from present legislation and new workable regulations are set up; (d) a realistic attitude toward the economy of exportation is maintained. F. W. BENNETT

353. New flavors for ice cream. F. W. BENNETT, University of Georgia, Athens. Southern Dairy Products J., 41, 2: 55. Feb., 1947.

Two new flavors for ice cream are described, muscadine and peanut butter. The muscadines are prepared by washing and removing foreign material which may be present. The grapes then are pressed to remove most of the pulp and juice, leaving hulls and seeds. The hulls are heated to tenderize and passed through a colander to remove the seeds, after which they are combined with the pulp and juice. One lb. of sugar is added for each 4 lb. of fruit. Two or 3 quarts of the sweetened fruit is sufficient for 10 gallons of ice cream. The sweetened fruit also may be stored indefinitely at 0° F. or lower.

Some brands of peanut butter are superior to others for use in ice cream.

Two lbs. of the product is the proper amount to flavor 10 gallons of ice cream. If the butter is mixed with 1 lb. of dry sugar before adding to the freezer, its even distribution will be facilitated. Both flavors have been well received by local consumers. F. W. BENNETT

354. Factory-produced carry-out sundaes. E. THOM, Assoc. Ed., Ice Cream Review. Ice Cream Rev., **31**, 11: 44, 45, 96. June, 1948.

The carry-out sundae, produced on a production line basis, is one means by which ice cream gallonage may be increased. At the Herron Ice Cream Company in Cleveland, a foursome package currently is being produced with considerable success. The package, approximately equivalent to 1 pint, consists of 4 ice cream rolls, approximately 2 inches in diameter, with a center core of fruit and pectin and a sprinkling of ground nuts, macaroons or almond bisque on the top.

A stainless steel extruder pipe is attached to the continuous freezer for producing the ice cream portion of the roll and the fruit is pumped to form the core through an inner stainless steel tube of any shape desired. Club, heart, diamond and spade combinations have proven to be the most popular core designs. The ice cream roll with its center core as it emerges from the 2 tubes, one inside the other, is fed onto stainless steel pans 3 ft. long. The pans move on a conveyor directly to the hardening room. Just before entering the hardening room, the pans pass beneath a device which sprinkles the top of the ice cream with ground macaroons or nuts, etc. After hardening, the ice cream roll is cut into any desired length. The individual portions then are placed into individual cellophane containers and packaged in units of 4 in cardboard boxes.

The package retailed for 35 cents in Cleveland at the time when ice cream was being sold for 30 cents a pint. This foursome package has proven extremely popular for sale directly into homes, and has enjoyed an excellent market among hotels, churches, lodges and other organizations holding large dinners. It has proven particularly helpful in keeping up sales during periods of low production.

The Esmond Dairy Company at Sandusky, Ohio, uses the same equipment to produce the sidewalk sundae. This item consists of a roll of ice cream with a fruit core, but without nuts or macaroons being added. The ice cream roll of suitable length is wrapped with paper, which is easily peeled off, and is then inserted in a cone. This item was introduced during the depression period to combat double- and triple-dipped cones.

W. J. CAULFIELD

355. The retail store of the future. D. GHORMLEY. Ice Cream Trade J., **44**, 6: 56. June, 1948.

The ice cream store of the future will be located in a suburban business

district within a few blocks of a middle-class residential district and on a main boulevard. It will be found in a specially constructed building, 35 × 60 feet, which will have a large plate glass window, a 12-foot ceiling in the store area, a mezzanine over the storage area and wash rooms for customers. The fixtures will cost between $7,000 and $9,000 and will be made of colored plastics and stainless steel. All food preparations will be done in the kitchen and picked up by waitresses through pass-out windows. Dish washing will be done in a separate room. The store will seat 37 to 43 and will have 4 booths.

Standardization of food preparations, salesmanship and the daily operating technics will be utilized. The company president will be an experienced retailer and constantly on the alert for new ideas. Many side-line products will be handled by the store of the future. The store will be air conditioned, and have an "electric eye" door and a concealed nickelodian. There will be no home delivery or credit offered. W. H. MARTIN

356. Borden's guides its dealers on retail selling prices. ANONYMOUS. Ice Cream Trade J., **44**, 6: 46, 82–84. June, 1948.

The Borden Company letter announcing price increases on ice cream was accompanied by a brochure entitled, "How to Price Ice Cream." Prices recommended for hand-dipped half-pints, pints and quarts were 25, 45 and 85 cents, respectively. For sodas costing 6.94 cents, a 15-cent selling price was suggested. Dealers were cautioned to price correctly and to watch for consumer reaction to selling price as a guide to greater sales.

W. H. MARTIN

357. Price trends. ANONYMOUS. Ice Cream Trade J., **44**, 6: 44, 45, 93–98. June, 1948.

Higher cost of dairy products has caused ice cream manufacturers to increase the wholesale price of bulk ice cream 10 to 15 cents per gallon and the price of packaged ice cream 15 to 25 cents per gallon. In the East, bulk vanilla ice cream is priced at $1.60 to $1.80 per gallon, with quantity discounts. In Chicago the price reported was $1.85 to $1.87 per gallon on bulk vanilla. Prices in the South, Oklahoma, California and Kansas range from $1.40 to $1.50 per gallon and in Minneapolis $1.20 to $1.30 per gallon. W. H. MARTIN

MILK

358. Frozen storage of milk as a method of preservation. F. J. DOAN, The Pennsylvania State College. Milk Dealer, **37**, 8: 44, 102–112. May, 1948.

A discussion of frozen concentrated skimmilk, frozen fluid whole milk

and frozen concentrated whole milk which the author summarizes as follows: Bulk frozen concentrated skimmilk is now being used, in a limited way, commercially for preserving milk solids-not-fat for later use, usually in ice cream. It has some advantages over other storage products and probably will be used more generally for this purpose. Another, frozen homogenized fluid milk, proved very satisfactory as a preserved substitute for fresh fluid milk for Army uses during the war where expense was not a limiting factor. It is unlikely, however, to become an important commercial article in the peace-time economy. The third, frozen concentrated whole milk can be employed for storing whole milk solids in bulk form and may at some future time appear on the retail market as one of the increasing number of frozen foods which can be held on hand in the home freezer or individual locker by the consumer. All three of these products are examples of an old method of frustrating nature's demolition corps, the microorganisms, but they represent new foods protected by such means.

C. J. BABCOCK

359. Borden introduces oblong 2-quart glass bottle. ANONYMOUS. Milk Dealer, 37, 8: 43. May, 1948.

Introduction of the space saving oblong two-quart glass milk bottle at food stores in Chicago and vicinity is announced by Borden's Chicago Milk Division. Thus, Chicago becomes the second city in the country to have this type of bottle, as it was introduced in New York some time ago.

The oblong bottle takes up 28% less space in a home or store refrigerator than the round half-gallon bottle and takes 33% less space than 2 single round quart bottles. The oblong container takes up 328 cubic inches of space as compared with 456 cubic inches for the round bottle. Another advantage of the oblong bottle is that it is easier to pour from. The oblong shape permits a firm grip on the sides. C. J. BABCOCK

360. Plant operation and efficiency. L. C. THOMSEN, University of Wisconsin. Milk Dealer, 37, 8: 47, 48, 140–148. May, 1948.

Under operating costs, it is pointed out that on the basis of best available averages it appears that presently most fluid milk plants will require 36 cents to 45 cents of every sales dollar for operation, reserves for depreciation and a reasonable return on a justifiable investment. Of this amount, well over one-half is paid out directly for wages and salaries. Executives with officer titles receive less than one-half cent of the sales dollar. Under the heading "Labor Savings" filling is pointed out as the key operation, and if it is not properly timed as to output, efficiency is inclined to suffer.

The following proposals for sales economies are given: (a) increase size of retail and wholesale loads by use of lighter containers and cases; (b) use

public utility types of practices to restrict areas of operation; (c) expand store sales, which have been shown to cut sales costs to less than one-half of those for retail delivery; (d) establish numerous milk or dairy stores; (e) still further curtailing services to the consumer by, for example, delivery every third day; and (f) offer quantity discounts.

In discussing ways of reducing milk losses, the author shows that total "paper" losses in ideally operating milk plants selling fluid milk and cream amount to approximately 1.65%, of which slightly less than three-fourths can be assigned to the fluid milk part of the operations, and slightly over one-fourth can be charged to the fluid cream part of the operation.

H.T.S.T. systems, proper routing of milk, methods of reducing waste and care of equipment are also discussed. C. J. BABCOCK

361. New products developments leading to greater milk sales. K. G. WECKEL, University of Wisconsin, Madison. Milk Dealer, **37**, 8: 50–56. May, 1948.

Preventing oxidized flavor, unsterilized evaporated milk, uniform equipment, extended delivery routes and electronic temperature recorders are discussed as potentialities that can improve and affect the sales and distribution of market milk. C. J. BABCOCK

362. The manufacture of a high quality chocolate milk drink. BENJAMIN P. FORBES, Benjamin P. Forbes Co., Cleveland, Ohio. Southern Dairy Products J., **44**, 1: 42–3. July, 1948.

Due to its greater miscibility, cocoa powder rather than chocolate is used in the preparation of chocolate milk drinks. The necessity for scrupulously observing the rules prescribed by the flavor manufacturer is obvious. In using reconstituted milks, the solids should be the same as in fresh milk.

Settling may be caused by: (a) insufficiently stabilized cocoa; (b) too much milk or insufficient butterfat content; (c) dilution of dairy drink by milk or water; (d) restriction of valves on pressure side of pumps, causing a homogenizing effect or excessive whipping by centrifugal pumps; (e) too acid milk (do not neutralize); (f) using soda-neutralized milk; (g) using frozen milk; (h) incorrect pasteurizing and cooling; (i) insufficient heat or too short holding time; (j) over-agitation during and after cooling; (k) powder not entirely incorporated; (l) not cooled to 40° or lower; (m) precooling below 140° in the vat.

Thickening may be caused by: (a) excessive pasteurizing temperature; (b) overstabilization; (c) use of lime or magnesium stabilizers; (d) improperly rinsed bottles; (e) milk which is too acid. F. W. BENNETT

363. Factors to consider in making high quality chocolate milk drinks. W. C. THACKER. Southern Dairy Products J., **43**, 2: 102–3, 116. Feb., 1948.

Chocolate milk is not consumed as a substitute for white milk. In 3 urban markets, the total per capita consumption of milk was 18% higher in the families that included chocolate milk.

The 6 desirable characteristics of chocolate milk or chocolate drink are: (a) High quality milk or part-skimmed milk. The minimum fat standard for whole milk should be met. (b) Mild chocolate flavor. The amount of flavor used ranges from 1.0 to 1.5% cocoa or 1.5 to 2.25% liquor chocolate. Cocoa is most used. Quality varies widely. Prepared syrups are convenient for small distributors, but the addition of the dry ingredients before pasteurization is less expensive. (c) Little or no sedimentation with a low to medium viscosity. Special finely-ground cocoas are on the market. Sodium alginate will prevent sedimentation at a low viscosity. Homogenization increases sedimentation. (d) Elimination of the ragged dark-colored cream layer. This can be prevented by pasteurization at 160 to 165° F. for 15 to 30 minutes and the addition of stabilizers. (e) Light to medium red-brown color. (f) Medium to high sweetness. Consumer demands differ from about 5 to 8% sugar. Standard composition and processing methods are important. F. W. BENNETT

364. Production can be leveled. H. F. SIMMONS, Michigan Milk Producers' Association. Milk Dealer, **37**, 8: 92-100. May, 1948.

A discussion is given of the "Base and Surplus Plan" as a means of leveling the production of milk by farmers so that it more nearly conforms to fluid milk requirements. C. J. BABCOCK

SANITATION AND CLEANSING

365. New sanitary practices and controls in the dairy industry. M. G. PEDERSON, Price's Creameries, Inc., El Paso, Texas. Southern Dairy Products J., **42**, 1: 34–5, 42–3. July, 1947.

Sediment may be received in the ice cream plant through milk and its products, sweetening agents, stabilizers and even the city water. In the plant, sediment may be introduced from solid flavoring materials, air in the plant, undissolved particles of cleaning and sterilizing agents, lubricants not held back by packing glands and cartons which have not been fully protected. Unsanitary practices of employees are another factor. Insects and rodents are important. Applying a 5% or stronger emulsion of DDT to the walls and ceiling every 2 or 3 weeks should control flies. Sodium fluoride, pyrethrum, rotenone and roach poisons, when regularly and prop-

erly used, are effective against cockroaches. Daily cleaning of the plant and the elimination of cracks and unused equipment which may harbor them are essential. Mouse-proof construction, trapping, poisoning and the elimination of rubbish are means of exterminating mice and rats. Antu, thallium sulfate, barium carbonate, zinc phosphide and strychnine should be used by an experienced and intelligent person. F. W. BENNETT

366. Cleaning and disinfecting milk and ice cream processing equipment. M. A. BAILEY, The Diversey Corporation, Chicago, Ill. Southern Dairy Products J., **41**, 4: 73, 78–81. Apr., 1947.

Clean-up operations consume over one-third of the labor in the dairy and therefore are worthy of serious consideration. In the 1920's, soap and washing soda were the two commonly used cleaning agents. Soap left a film of soap or scum on the equipment, did not possess the necessary cleaning power and was expensive to use. Action on the hands accompanied the use of washing soda, lime films were left and its cleaning performance was still far from efficient. Chemists set out to blend the various sodas with trisodium phosphate and metasilicate to obtain balanced cleaners.

In selecting the product to be used for general cleaning, the cleaner must remove contamination quickly and safely; no dirt or deposit must be left on the equipment; the cleaner must not damage the equipment; the cleaner must not be hard on the hands of the operator and must be liked by him; it must be economical to use judged by the results obtained.

There are six essential characteristics that a good cleaning compound should exhibit: water softening to eliminate a mineral build-up on the equipment; wetting action to penetrate and wet the film to be removed; suspending action to prevent the settling out of soil to re-adhere to the equipment; emulsifying ability to keep fats suspended and allow them to be rinsed off easily; rinsing ability so that the cleaner itself may be easily removed; bactericidal action in special operation such as bottle washing.

Milkstone may be removed safely and prevented by the successive use of specially prepared acid and alkaline cleaners in short-time pasteurizers, vacuum pans and evaporators.

Training of the workers in the care and cleaning of equipment is of the utmost importance. The importance of their work must be impressed upon them for best results. Trained representatives of several chemical companies will assist gladly in an educational plan for plant employees.

F. W. BENNETT

MISCELLANEOUS

367. Refrigeration for dairy plants. A. A. GEIGER, York Corporation, Atlanta, Ga. Southern Dairy Products J., **39**, 5: 82, 88–9. May, 1946.

The refrigeration for dairy plants should be designed to handle easily

the maximum load, require no hand regulation of control valves for minimum loads and have sufficient flexibility. All efforts should be made to make the plant free of shut-down possibilities. There are 3 types of refrigerating mediums for cooling milk products: brine, direct expansion, or sweet water. For the last 10 years, milk machinery manufacturers of vertical vats with spray jackets have tended to recommend cooling by sweet water rather than brine. If an open type sweet water tank is used, an ice field is built up during the night to serve as a storage of refrigeration. As the ice melts during milk cooling, the temperature of the sweet water reaching the milk cooler may increase 20°. For this reason, this system cannot be recommended for milk plant use. The shell and tube water cooler to deliver 34° F. water is subject to freezing up. Instantaneous water coolers with controls to deliver water at uniform optimum temperature are available. Brine for cooling makes it possible to use a smaller milk cooler because of a lower brine temperature. When brine is used, only one ammonia control is necessary. The compressor in a brine system can be operated at 100% capacity. The wide range of temperature possible in the brine enables the plant to have a low enough temperature at all times.

Corrosion should not be a problem if the brine is not allowed to become acid. Brine may be used in a plate cooler if the tightening head is equipped with a compensating spring and a proper brine control is used. The direct expansion system is not advisable for small plants because of (a) excessive cost on small volumes, (b) complicated controls and (c) short-cycling of compressors. This system adapts itself advantageously in larger plants, but for buttermilk and cream cooling, the sweet water system is used.

A two-compressor installation is recommended rather than a single compressor for flexibility. Too few operators of milk plants devote enough attention to their refrigeration needs. F. W. BENNETT

368. How employer-employee relationships reflect good management. J. W. POST, Armour and Co., Chicago, Ill. Ice Cream Rev., **31, 11**: 76, 78, 80, 82, 84. June, 1948.

The losses or gains as a result of customer satisfaction or dissatisfaction depend upon the kind of personnel developed by the company. Management must assume the responsibility for seeing that the right kind of personnel is developed, as only a few good employees are self-made. Fourteen groups of desirable traits and habits which should be developed in employees are enumerated.

Better employer-employee relationship may be developed in many ways. Some of the methods currently used by well-managed businesses include the following: employee publications to improve the line of communications between employees and the company; job evaluation as it applies to wage

rates to prevent inequities; polling of employees for opinions, ideas and suggestions for improvement; regular training and educational procedure which is oral, visual and written; aptitude and psychological testing that help in employee selection and in job assignments; periodic merit rating by procedures which meet the need for individual appraisal; provision for recreational and health protection facilities; and posting of employment policies.

Another factor to be considered is that of federal and state legislation as it affects employer-employee relationships. Plans must be formulated to meet such things as sick-benefit plans, life, health and accident plans, profit-sharing or merit-rating plans. The position of management with respect to labor relations is entirely different from that of a few years ago. Business now is confronted with inflation, full employment and much new legislation which affects employer-employee relations.

Management must devote more time to the personnel problem. What your employees think and express determines in a large measure the public relations of the company concerned. How well the employer-employee relationship problem is solved will determine in no small measure the success of a given business under present-day conditions. W. J. CAULFIELD

369. Economic aspects of open and closed cream markets. LELAND SPENCER, Cornell University. Milk Dealer, **37**, 8: 86–88. May, 1948.

Following a listing of the consequences of maintaining strict requirements as to conditions under which cream is produced and handled and also limiting the area of inspection, it is concluded that: (a) the "closed" market policy for cream does not result in higher cost to consumers for fluid milk; (b) the extra income for producers is obtained through higher prices for cream than would prevail in an "open" market; (c) the extra cost of cream is shared by consumers and distributors of fluid cream and sour cream and by manufacturers of ice cream.

Another point worthy of emphasis is that the milk produced for use as cream in a "closed" market is a reserve supply that can be drawn upon for use as fluid milk whenever the milk supply from inspected sources is insufficient for all demands.

The advantages and disadvantages of requiring cream to be produced and handled under strict sanitary requirements within a limited production area are difficult to evaluate. Considerations of public health and aesthetics as well as economics are involved. There is need for a thorough and careful determination and appraisal of pertinent facts as a basis for intelligent action. C. J. BABCOCK

370. Trend toward conversion to oil as fuel continues. ANONYMOUS. Milk Dealer, 37, 8: 40. May, 1948.

A survey made by the Olsen Publishing Co. of milk dealers in New England and North Central states shows that the trend among milk dealers toward converting plants from coal to oil is continuing.

Out of 434 replies received to questionnaires, 54.5% reported they are now using oil as fuel, 43.4% are using coal, and 3.1% are using gas. Among these same dealers, however, 14.1% indicated they are planning to make a change in the type of fuel used, and of these, 71.5% expect to change from coal to oil and another 10.7% from coal to gas.

The determining factor mentioned most often by respondents to the questionnaire was that oil or gas was cleaner than coal. Second most popular determining factor was that oil or gas saves labor; third was convenience; fourth was price; and fifth was efficiency. C. J. BABCOCK

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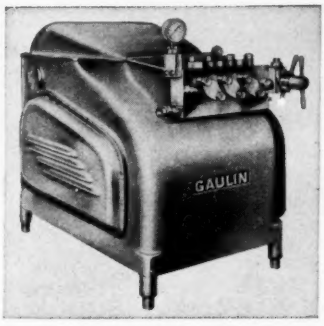

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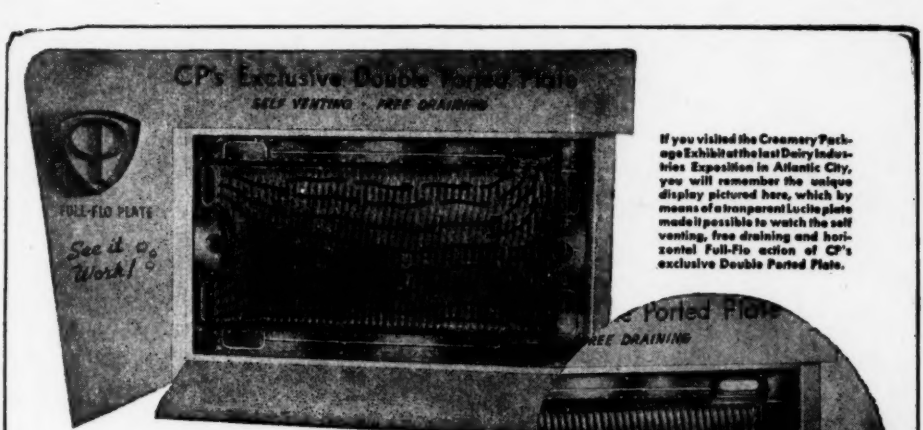

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